Molecular detection of microbes

P941

Direct detection of *Salmonella* spp. in faecal specimens by real-time PCR assay

I. Pachiadakis, V. Karavassilis, A. Vasdeki, A. Melidou, A. Goudouva, P. Lisgara, E. Petinaki, A. Maniatis *(Larissa, GR)*

Objective: To evaluate a commercial kit for the detection of *Salmonella* spp. in faecal specimens based on Real-Time PCR (Real-Art TM *Salmonella* RG PCR Kit).
Methods: A total of 1000 faecal specimens obtained consecutively from individual patients of Thessalia (Central Greece) were included in this study. Genomic DNA extractions were performed by use of commercially available isolation kits, while the commercial kit described above, according to the instructions of the manufacturer did real-time PCR. Results obtained from Real-Time PCR assay were compared with bacterial culture results to determine sensitivity and specificity.
Results: According to the results obtained by Real-Time PCR, fifty-three of 1000 faecal specimens were positive for *Salmonella* spp. *Salmonella* was detected by bacterial cultures in all, except two, the positive specimens. Repetition of two negative cultures after enrichment detected the pathogenic micro organisms. The mean time for the detection of *Salmonella* by Real-Time PCR was approximately 5–6 hours, while detection by traditional culture methods required 2–3 days.
Conclusion: The high sensitivity and specificity of this Real-Time PCR assay emphasizes the need of its application in a routine clinical laboratory for the rapid and accurate detection of *Salmonella* in faecal specimens.

P942

Multicentric evaluation of Onychodiag® for diagnosis of dermatophyte onychomycosis by PCR-ELISA

C. Savin, S. Huck, C. Rolland, M. Benderdouche, O. Faure, G. Noacco, J. Menotti, E. Candolfi, H. Pelloux, R. Grillot, F. Derouin, S. Coupé *(Emerainville, Strasbourg, Grenoble, Paris, FR)*

Objective: To prospectively assess the performance of a PCR-ELISA assay (Onychodiag®, Bio Advance, France) for diagnosis of onychomycosis due to dermatophytes.
Patients/Methods: Three-university hospital labs specialized in medical mycology participated to the study. In each lab, nail samples (NS) were collected from patients (pts) suspected for onychomycosis (n = 404) or healthy controls (n = 108). In labs A and B, trained mycologists taking care to collect the sample as close as possible to the lesion collected NS. In lab C, NS were collected by other physicians and referred to the lab. Each NS was processed by classical mycological techniques i.e. direct examination (DE) and culture (Cult) and tested by Onychodiag® blindly to the results of mycology. For 75 pts, an additional distal sample (DS), obtained by clipping the nail plate, was collected from the same dystrophic nail and tested by Onychodiag® only, as such sample is improper for mycology. Onychodiag® processed each NS as follow: DNA extraction, amplification by PCR, hybridization with a labelled dermatophyte-specific probe and immobilization in a microplate well recoated with a capture probe. Results were expressed as OD values and considered positive for OD > 0.5, negative for OD < 0.3 and borderline (grey zone) for 0.3 < OD < 0.5.
Results: See table In pts with a culture proven onychomycosis due to a dermatophyte, the sensitivity of Onychodiag® was 75, 83 and 100% for lab A, B and C respectively (mean 82%; 87% including grey zone). The specificity was 98% when considering healthy subjects as true negative controls. In pts with suspected onychomycosis but with negative culture for dermatophyte, Onychodiag® was positive in 52% of pts with positive DE and 18% of pts with negative DE. Based on the results of Onychodiag® on mycological proven onychomycosis and healthy controls, these results were considered as true positive. The low performance of mycology on these samples was related to non-proper conditions of sampling. For DS, mycology results on properly collected samples (same nail) were used as reference. Onychodiag® on these samples was positive in 49/53 (92%) cases of proven dermatophytic onychomycosis.

	No.	Onychodiag® (no., %)		
		Pos	Neg	Grey zone*
Culture proven dermatophyte onychomycosis	131	108 (82%)	17 (13%)	6 (5%)
Healthy controls	108	2 (2%)	106 (98%)	0
DE Pos and Cult Neg (or pos. for yeasts or moulds)	52	27 (52%)	18 (35%)	7 (13%)
DE Neg and Cult Neg (or pos. for yeasts or moulds)	146	26 (18%)	111 (76%)	9 (6%)
Distal samples and Cult Pos for dermatophyte on correct NS	53	49 (92%)	0	4 (8%)
Distal samples and Cult.Neg for dermatophyte on correct NS	22	6 (27%)	16 (73%)	0

* bordeline value of ELISA

Conclusion: Onychodiag® provides diagnosis of onychomycosis due to dermatophytes (without species identification) within 24–48 hours after sampling. Its sensitivity on properly collected samples and on DS was close to that of mycological techniques performed on properly collected samples.

P943

Validation of PCR-RFLP analysis of the gap gene as a useful tool for the species-level identification of staphylococcal isolates

S. Grzeszczuk, A. Sabat, M. Bartoszewicz, A. Przondo-Mordarska, W. Hryniewicz *(Warsaw, Wroclaw, PL)*

Objective: An increase in the number of infections due to coagulate negative *Staphylococcus* (CoNS) has been documented. Therefore, rapid and accurate identification of CoNS species may provide important diagnostic information, which would allow the selection of an appropriate course of treatment in a timely and effective manner. The gap gene encoding glyceraldehyde-3-phosphate dehydrogenase has been proposed as a target for the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis for identification of 24 Staphylococcus species. The goal of this study was to evaluate the reliability of the PCR-RFLP system in the identification of staphylococci at the species level.
Methods: A collection of 202 mostly clinical as well as laboratory strains comprising 30 staphylococcal species was selected. Isolates were identified to the species level by partial sequence analysis of the 16S rRNA, sodA and rpoB genes; PCR-based amplification of the gap gene followed by RFLP analysis with restriction enzyme *Alu*I; and biochemical tests based on ID 32 STAPH (bioMerieux) and the presence of clumping factor and coagulase.

Results: Using the PCR-RFLP method, we examined the polymorphism of gap gene. During this study we determined the gap genotypes by AluI PCR-RFLP for further six staphylococcal species, therefore we were able to increase the list of species from 24 to 30. PCR-RFLP results were consistent with the results of partial sequencing of 16S rRNA, sodA and rpoB. Biochemical tests agreed in only 86% with other methods tested.
Conclusion: These results suggest that the PCR-RFLP assay provides rapid, accurate, and reliable species-level identification of staphylococci. By using a series of control strains in each experiment the assay will be easy to standardise and interpret.

P944

Clonal complexity in coagulase negative *Staphylococcus* catheter-related bloodstream infection

C. Aldea-Mansilla, D. García de Viedma, E. Cercenado, E. Bouza, M. Ma rín *(Madrid, ES)*

Objective: To determine the clonal features of catheter-related bloodstream infections (CR-BSI) by Coagulase-Negative *Staphylococcus* (CNS) and to evaluate the application of molecular tools in the CR-BSI diagnosis.
Methods: We studied 61 CNS CR-BSI episodes from 55 patients with CNS cultured from peripheral blood and catheter tip cultures. All different CNS morphotypes (colonies with different morphology) were picked from each culture, and biotype, antibiotype and genotype (PFGE type) were obtained.
Results: Morphological differences were correlated with differences in the PFGE type in 71% of the cases. Among these, 98% correlated with differences in antibiogram, 61% in biotype and 14% at species level. Simultaneous presence of more than one CNS clone (polyclonality) was found in 17% of catheters and in 44% of bloodstream infections. Only 77% of the CNS CR-BSI suspected cases were confirmed by molecular methods and 44% of true CNS CR-BSI episodes had not been diagnosed if only a single colony had been randomly selected from blood and catheter tip cultures. In 5% of the molecularly confirmed CR-BSI, two different CNS clones were tracked in catheter tip and blood cultures and they were the cause of polyclonal CR-BIS. In half of the true CR-BSI episodes, the CNS clone involved in CR-BSI was accompanied by other CNS clones, and in 24% CNS CR-BSI this clone was minority found in blood cultures (less than 50% of the isolates involved). Epidemiological study revealed 105 clones, grouped in 35 mayor clusters of isolates (from 1 to 8 clusters) with > 80% homology. There was no greater presence of any clone in any of the hospital services or during any particular month.
Conclusion: Molecular tools allowed revealing the microbiological complexity of CR-BSI by CNS. A precise analysis and diagnostic of CNS CR-BSI requires to consider this finding and to consequently redefine the microbiological procedures and tools to efficient management of this entity.

P945

Effect of treatment on *Toxoplasma*-specific IgG antibodies and IgG avidity maturation

V. Meroni, F. Genco, G. Ferraris, L. Bollani, L. Piccoli, C. Bodini, P. Lanzarini *(Pavia, Milan, IT)*

Objective: Aim of the study was to evaluate the influence of treatment with spiramycin on the increase of IgG titre and IgG avidity index in pregnant women with seroconversion or very recent *Toxoplasma gondii* infection (twofold or greater IgG antibody increase, IgM positive, low avidity index), from beginning of therapy until delivery, in comparison with adult patients with recently acquired, but not pharmacologically treated, toxoplasmosis.
Methods: Eighteen pregnant women with seroconversion for toxoplasmosis and/or very low IgG avidity index were followed, from beginning of therapy with spiramycin, until delivery. Fifteen out of 18 pregnant women were also tested a few months after delivery and interruption of therapy. IgG antibody response and IgG avidity index were also evaluated in a control group of 11 adult patients with seroconversion or very recent infection. All serum samples were tested with commercially available kits: VIDAS TOXO IgG II, VIDAS TOXO IgG AVIDITY, Toxo-ISAGA (bioMerieux, Marcy l'Etoile, France), LIAISON Toxo IgG II and LIAISON Toxo IgG Avidity II (DiaSorin, Saluggia, Italy).
Results: Maturation of *Toxoplasma*-specific IgG antibodies and IgG avidity are delayed after therapy administration in pregnant women. When we compared the time curves obtained with untreated patients, we observed faster maturation of IgG and IgG avidity index in the latter ones. In addition, an antibody rebound was observed in the treated pregnant population with IgG avidity maturation after delivery and interruption of therapy.
Conclusion: As previously described, therapy with spiramycin may modify IgG antibody response and IgG avidity maturation in pregnant women. The reason for the delay in serological response may be reduction of parasitic load in early treated infections due to the effect of spiramycin on trophozoite stage. The antibody rebound observed after delivery and therapy interruption may thus also be explained.

P946

Evaluation of rapid molecular methods in diagnosis of B streptococci under delivery

B. König, B. Ghebremedhin, W. König *(Magdeburg, DE)*

Objectives: Group B streptococcal infections are a leading cause of neonatal mortality. To prevent the severe diseases, especially the "early onset disease", either a risk-based or a screening-based approach is used to identify candidates for intrapartum antibiotics. In the latter case all women are screened for carriage of group B streptococci between 35 and 37 weeks of gestation by culture, and intrapartum chemoprophylaxis is offered to carriers. However, the culture strategy may not accurately predict genital tract colonization at time of labour and potential over treatment as well as under treatment is an issue of debate. There our study looked at a new rapid polymerase chain reaction (PCR) assay as an alternative-screening tool.
Methods: We evaluated a fast polymerase chain reaction test, the IDI-GBS® (Cepheid), for rapid identification of B streptococci colonized women ad admission for delivery. In this rapid PCR technique, a sample (genital/anal swab) is analysed using fluorescent hybridization probes and fluorescence detection and results are available in less than 1 hour.
Results: During a two months period we analysed 94 swabs (vaginal/anal) from women under delivery for the presence of B streptococci by culture and PCR (IDI-GBS®) in parallel. The results from both methods were 100% congruent. B streptococci were detected in 13 cases (13.8%). All B-streptococci were penicillin susceptible.
Conclusion: One of the potential advantages of using this test for screening is that it could be used at time of admission for labour, therefore offering better sensitivity and specificity for predicting colonization in women at the time of labour. Furthermore, mothers who did not receive prenatal care could be screened with the PCR technique at time of admission to the hospital and rapid processing makes it possible to admister chemoprophylaxis promptly to women before delivery.

Screening with the convential culture, which takes 2 or 3 days, would not be possible for such women.

P947

Diagnosis of human brucellosis using AMOS PCR

W. Yu, K. Nielsen, T. Grushina, T. Meka-Mechenko *(Nepean, CA; Almaty, KZ)*

Objectives: Numerous PCR-based assays have been developed for the identification of *Brucella* to improve diagnostic capabilities. For some purposes, the simple identification of *Brucella* is adequate. However, PCR with IPS1 and IPS2 primers is a reliable tool for rapid identification of *Brucella* species and its differentiation from closely related organisms. A fundamental question that arises during epidemiological investigations of outbreaks is whether the outbreak strain is genetically related to a proposed strain. Highly discriminating genetic markers for characterizing bacterial strains can help in clarifying the genetic relationships among strains. The *Brucella* AMOS (AbortusMelitensisOvisSuis)-PCR assay was previously developed to identify and differentiate specific *Brucella* species.
Material and methods: Twenty strains of *B. melitensis* isolated from humans and animals using traditional techniques during four field trips to rural areas in two regions of Kazakhstan. Total 360 people were examined. *B. melitensis* biovars 1, 2 and 3 were isolated in 18 cases out 130 individuals presumed to be infected with *Brucella* sp. Two *B. melitensis* biovar 2 were isolated from sheep milk. Twenty isolates were identified and tested by the conventional biochemical tests, PCR-based and *Brucella* AMOS PCR
Results: Twenty isolates were identified and tested by the conventional biochemical tests, PCR-based and *Brucella* AMOS PCR. This included 6 isolates identified as *B. melitensis* biovar 1; 10-identified as *B. melitensis* biovar 2; 4-identified as *B. melitensis* biovar 3. The results of classical typing for 20 *B. melitensis* cultures isolated from humans and animals were confirmed at genetic level by PCR. The high specificity of this PCR-based assay was demonstrated by testing all of the biovars of twenty strains of *B. melitensis*. The Brucella AMOS PCR correctly identified each isolate as a *B. melitensis*. However due to AMOS, but not biological typing, genetic features of 2 strains of *B. melitensis* biovar1 and 2 have been found. Two *B. melitensis* biovar 1 and 2 have been isolated from the people with serological positive acute brucellosis (SAT: 1: 1600).
Conclusion: The PCR-based test and AMOS-PCR could be used as a complementary test to identify genus *Brucella* and differentiate specific *Brucella* species. However, to explain further genetic distinctions, fingerprinting is required.

P948

Evaluation and comparison of molecular tests for identification of methicillin-resistant *Staphylococcus aureus*

A. Kerttula, A. Vainio, S. Mero, T. Pasanen, J. Vuopio-Varkila, A. Virolainen *(Helsinki, FI)*

Objective: The aim was to compare different in-house and commercial molecular methods to detect methillicillin-resistant *S. aureus* (MRSA).
Methods: A total of 208 bacterial isolates were tested: 122 consecutive, clinical *S. aureus* strains [108 MRSA, 7 borderline methicillin-resistant *S.aureus* (BORSA) and 7 methicillin-sensitive *S.aureus* (MSSA)] sent to National Public Health Institute for confirmation and genotyping during a two month period, 19 Finnish epidemic MRSA (EMRSA) strains, 2 BORSA, 1 MSSA, 3 ATCC *S. aureus* reference strains, 41 *S. epidermidis* strains, 18 other ATCC staphylococcal reference strains, 1 *Enterococcus faecalis*, and 1 *E. faecium* isolate. We compared Geno Type® MRSA test (detection of mecA, universal bacterial control, *S. aureus* and *S. epidermidis* specific DNA), EVIGENE™ test (mecA, nuc, 16SrRNA present in all staphylococci), LightCycler MRSA Detection Kit (mecA) and in-house real-time-PCR method (RT-PCR) (mecA, nuc) to conventional in-house PCR of mecA and nuc test.
Results: All 144 *S. aureus* isolates tested positive for the nuc- and 127 for the mecA-genes in our in-house PCR-tests. The results obtained with Geno Type® MRSA, i.e. detection of mecA and amplicons specific to *S. aureus* species, corresponded with the conventional PCR results. The Geno Type® MRSA also correctly detected all 41 *S. epidermidis* strains, of which 39 possessed the mecA-gene. Both Enterococcus isolates and all other coagulase-negative staphylococcal isolates remained negative, except for one strain possessing the mecA gene. EVIGENE™, in-house RT- PCR and LightCycler MRSA Detection Kit test results corresponded with the conventional PCR results with all 127 mecA-positive MRSA strains. MSSA and BORSA strains were detected correctly also by using EVIGENE™ but the real-time PCR methods gave some false positive or uncertain results.
Conclusion: A good variety of reliable commercial molecular tests for rapid MRSA identification and confirmation were available for clinical microbiological laboratories.

P949

Comparison of fluorescent *in situ* hybridization and histological method for diagnosis of *Helicobacter pylori* in gastric biopsy samples

S. Tajbakhsh, A.R. Samarbaf-Zadeh, S.M. Moosavian *(Bushehr, Ahwaz, IR)*

Objective: The objective of this study was to compare fluorescent *in situ* hybridization (FISH) with histology for detection of *H. pylori* in gastric biopsy specimens.
Methods: Fluorescent–labelled oligonucleotide probes that target ribosomal RNA, were utilized in FISH procedure. In this project, FISH tested 91 specimens and histological method using the haematoxylin-eosin (H-E) and *Geimsa* stains. Furthermore, clarithromycin resistance in 39 samples of above 91 specimens were examined by FISH
Results: The sensitivity and specificity of FISH for detection of *H. pylori* were 97.9% and 97.7%, respectively. Of those 39 samples that were tested for clarithromycin resistance, 19 were FISH positive for *H. pylori*, which 15 and 4 specimens were infected with clarithromycin susceptible and clarithromycin resistant strain, respectively.
Conclusion: FISH is a highly sensitive and specific technique for diagnosis of *H. pylori* in gastric biopsies. The ability of FISH for detection clarithromycin resistant and susceptible strains is the essential advantage of this technique on histology.

P950

Comparison of a real-time PCR assay and a chromogenic MRSA selective medium for the detection of methicillin-resistant *Staphylococcus aureus* directly from nasal and rectal swabs

R. Reyes, B. Catanzariti, E. Isaac, M. John, L. Stoakes, J. Vandierendonck, Z. Hussain *(London/Ontario, CA)*

Objective: IDI-MRSA assay (Genome, California), a real-time PCR and *in vitro* diagnostic test amplify a highly conserved

MRSA-specific gene sequence within SCCmec and orfX. SCCmec is the mobile genetic element, which harbours the mecA gene. We evaluated the assay for the detection of MRSA from nasal and rectal swabs obtained from hospitalised patients and compared it with a chromogenic MRSA selective medium.
Methods: Copan swabs with liquid Stuart media were used to collect nasal and rectal specimens. For the IDI-MRSA assay, nasal swabs were processed as described by Warren *et al.* (J. Clin. Microbiol. 2004, 42: 5578). Rectal swabs were vortexed with 1 ml of sample buffer, 50 ml of which was added to lysis tubes. After brief vortexing of tubes, tubes were heated at 95°C for 2 minutes. Subsequently, 2.8 ml of cooled sample buffer was added to the PCR master mix. MRSASelect (Marnes la Coquette, France) plates were either inoculated directly with the nasal swabs or with 100 ml of the sample buffer prepared with rectal swabs.
Results: In total 259 pairs of nasal and rectal swabs were tested. Results of culture and IDI MRSA assay are shown below. Of 17 culture positive and PCR-negative results, 6 could be considered being true positive because MRSA was isolated from a subsequent nasal or rectal specimen. The positive predicative value increased to 78%, when 6 results were regarded as true positive. Thirty-one rectal and 21 nasal specimens could not be evaluated or to be repeated because of inhibition of amplification reaction.

MRSASelect	PCR positive	PCR negative	% Sensitivity	% Specificity	PPV%	NPV%
Nasal culture						
positive	19	1	95	94	58	99
negative	14	225				
Rectal culture						
positive	14	3	82	99	82	82
negative	3	239				
Total						
positive	33	4	89	96	66	99
negative	17	464				

Conclusion: IDI-MRSA assay is rapid and sensitive MRSA detection method from nasal and rectal specimens. The assay has advantage over other real-time PCR and conventional PCR methods that it can be performed directly on patient specimens and allows same day result. Disadvantage of IDI-MRSA include the high initial investment for equipment, and high cost of testing. We also observed a higher rate of inhibition than previously reported.

P951

Evaluation of SeptiFast – a new commercially available broad-range real-time PCR assay for detection of bacteria and fungi in blood

G. Lisby, H. Westh *(Hvidovre, DK)*

Introduction: Blood culture (BC) is the gold standard for detection of bacteraemia and fungaemia but has several limitations. False positive results due to contamination and false negative results due to e.g. antibiotic treatment at the time of sampling are well known problems. Nucleic acid target amplification (PCR) could potentially supplement traditional BC.
Objective: To compare a new commercially available broad-range real-time PCR assay (SeptiFast) with BC for detection of bacteria and fungi in blood from patients with suspected sepsis or SIRS. SeptiFast detects a total of 23 pathogen species.
Methods: A BC sample (32–40 ml) was obtained using sterile technique. Immediately after, a 5 ml blood sample was obtained for PCR. At the Department of Clinical Microbiology, Hvidovre Hospital, BC (BactAlert, BioMerioux) was performed according to standard operating procedures. PCR (SeptiFast, Roche Diagnostics) was performed according to the manufacturers' description using a MagNA Lyser and LightCycler 2.0 (Roche Diagnostics).
Results: A total of 114 episodes (combined BC samples and PCR samples) were collected from 114 patients. 83 episodes were negative in both BC and PCR and 21 episodes were positive in one or both assays. 13 episodes were BC positive, yielding 13 BC isolates (positive rate for non contaminant BC: 7.9%). Two isolates were not included in the PCR panel and three isolates were considered as contamination. Of the remaining seven isolates available for direct comparison, PCR detected five. 15 episodes were PCR positive (positive rate for non contaminant PCR: 11.4%), detecting a total of 19 microorganisms. Two of the detected microorganisms were considered as contamination, six were also detected by BC, but 11 were not detected by BC. Of these 11 PCR ± BC- isolates, six were confirmed by culture of the same micororganism from a clinical relevant anatomical site and five could not be confirmed by culture. The combined tests had a positive rate for non-contaminants of 15.8%. In the six episodes positive by both PCR and BC, PCR would reveal species identification in approx 18 hours if performed on a daily basis. BC had an average delay of 2 days until Gram stain, and 3–4 days until species identification.
Conclusion: This study shows SeptiFast PCR to be a valuable add-on to conventional BC for detection of bacteria and fungi in blood.

P952

Multiple aetiology of urethritis and cervicitis in sexually transmitted disease clinics caused by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis*

C.A. Gaydos, N.E. Maldeis, A. Hardick, J. Hardick, M. Barnes, B.J. Wood *(Baltimore, US)*

Objective: To determine the prevalence and multiple aetiologies of male urethritis and female cervicitis of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), *Mycoplasma genitalium* (MG), and *Trichomonas vaginalis* (TV) in patients from STD clinics using Gen-Probe APTIMA TMA and PCRs.
Methods: Urines from 290 men and self-administered vaginal swabs from 325 women were collected. Demographics, behavioural risk factors, signs and symptoms were recorded. CT and GC were detected by APTIMA Combo 2 (Gen-Probe Incorporated). MG and TV were detected by real time research PCR (MLRT-PCR for MG and B-TUB FRET for TV) and by prototype Gen-Probe TMA-based assays for MG and TV (TMA-MG and TMA-TV).). A patient considered to be infected with MG and/or TV if both research PCR assay and prototype Gen-Probe assay were positive.
Results: Prevalence: males (N = 153) with urethritis: CT 32.7%, GC 24.2%, TV 5.2%, MG 21.7%; males (N = 137) without urethritis: CT 6.6%, GC 0%, TV 1.5%, MG 8.0%. Prevalence: females (N = 77) with cervicitis: CT 18.2%, GC 7.8%, TV 18.2%, MG 26.0%; females (*N* = 248) without cervicitis: CT 9.3%, GC 3.2%, TV 13.8%, MG 17.5%. Kappa statistics for comparison between research PCRs for MG and TV and TMA-MG and TMA-TV were excellent (0.941 and 0.858, respectively). Co-infections of > 2 organisms occurred in 10.6% of males. Co-infections with > 2 organisms occurred in 11.4% of females.
Conclusion: Prevalence of all organisms was higher in men with urethritis and in women with cervicitis in this population. Co-infections were common. MG and TV were associated with urethritis and cervicitis. Prototype TMA-based assays for MG and TV performed very well compared to research PCR assays.

P953

Advances in diagnostic testing of *Chlamydia trachomatis*

M. Eickhoff, S. Cramer, T. Grewing *(Hamburg, DE)*

Objective: The genome of the obligate intracellular bacterium *C. trachomatis* consists of a circular chromosome of 1.045 MB and a conserved cryptic plasmid, which is approximately 7.5 KB in size and is present in multiple copies (5–10) in the organism. The plasmid has great practical importance as favoured target for nucleic acid amplification technologies (NAATs). However, a few isolates of *C. trachomatis* have been described, which do not contain the plasmid. We evaluated the performance of the artus™ *C. trachomatis* Plus RG PCR Kit (QIAGEN Diagnostics GmbH, Germany) which combines highly sensitive detection of chlamydial DNA from swab, urine and sperm samples and high specificity by using a combined *omp*A- and cryptic plasmid-based PCR.
Methods: The artus™ *C. trachomatis* Plus RG PCR Kit constitutes a ready to use system for the detection of *C. trachomatis* DNA by real-time polymerase chain reaction (PCR) using the Rotor-Gene™ instrument (Corbett Research, Australia). The *C. trachomatis* Plus RG Master contains reagents and enzymes for the specific amplification and the direct detection of a 106 bp region of the *C. trachomatis* genome and of a 111 bp region of the chlamydial cryptical plasmid. In addition, it contains a second amplification system (internal control) to identify possible PCR inhibition and to control the isolation procedure. Swab and sperm specimens were purified using the QIAamp® DNA Mini Kit, urine specimens using the QIAamp® Viral RNA Mini Kit (QIAGEN, Germany). The evaluation of the assay was performed with 142 retrospective swab samples (50 urethral, 48 cervical and 44 vaginal swabs), 94 retrospective urine samples (49 male, 45 female) as well as 65 prospective sperm samples.
Results: Diagnostic sensitivity and specificity for sperm sample have shown to be 100%. For swab and urine samples the artus™ *C. trachomatis* Plus RG PCR Kit showed a diagnostic sensitivity of 98% and a specificity of 99%.
Conclusion: Our results show that the new real-time PCR assay provides a sensitive and specific way to detect *C. trachomatis* in various sample materials. With the artus™ *C. trachomatis* Plus RG PCR Kit laboratory physicians no longer need to implement confirmation-PCRs that are necessary if commercial test systems are used, which are based on the cryptic plasmid only.

P954

LCR method for detection of *Chlamydia trachomatis* in patients with chronic nonbacterial prostatitis

N. Draskovic *(Belgrade, CS)*

Objective: Chronic prostatitis is frequent in men of all ages. Almost 95% of all prostatitis cases consist of chronic non-bacterial prostatitis (CNBP), which has unclear aetiology. In recent years role of *Chlamydia trachomatis* (CT) in etiopathogenesis of CNBP came into focus. Genital chlamydia infection is widespread and frequently asymptomatic and chronic, therefore some forms of prostatitis can be considered to be complication of genital CT infection. Aim of our study was to determine whether CT infection could be correlated to some CNBP forms.
Method: Samples of 72 patients (32 in CNBP group and 41 with prostatodynia as a control group) were analysed. Sampling was performed according to standard Starney and Mears method (urethral swab, VB1- first urine flash, VB2-middle urine flash, EPS - expressed prostatic secretions, VB3-urine after prostate massage). This kind of sampling enables segmental localization of infection. Samples were analysed for bacteria, CT determination was done by nucleic acid amplification performed by ligase chain reaction (LCR). Direct microscopy of Gram-stained prostate experiment was performed. Infection criterion was finding of more than 10 white blood cells (WBCs) per high-power field (HPF).
Results: In CNBP group, there was no CT in urethral swab and VB1. In VB2 samples CT was found in 1 (3.13%), in EPS samples in 7 (21.88%) and in VB3 samples in 5 (15.63%). In control group with prostatodynia, CT was found only in one EPS sample (2,44%). In all other samples CT was not present.

Samples	Positive result of CT (LCR)	
	Group 1	Group 2
	n = 41 N (%)	n = 32 N (%)
Urethral swabs	-	-
VB1	-	-
VB2	1 (3,13%)	-
EPS	7 (21,88%)	1 (2,44%)
VB3	5 (15,63%)	-
Total	13 (40,64%)	1 (2,44%)

Conclusion: In CNBP group, CT was determined in samples by LCR in 40.64% and in control group in 2.44%. Difference is statistically significant. In CNBP patients who were CT positive (13), EPS sample was positive in 7 (53.08%). CT can be correlated with some forms of CNBP, but for valid assessment sampling must be done according to Stamey and Mears method.

P955

Improvement of an existing magnetic particle based NAAT sample preparation system for *C. trachomatis* in urine by significantly reducing processing time while maintaining sensitivity

M. Anglés d'Auriac, T. Engen, U.H. Refseth *(Oslo, NO)*

Objective: *Chlamydia trachomatis* is the leading cause of sexually transmitted disease worldwide and is still on the rise. To obtain control over this pandemic, health authorities have been promoting the use of non-invasive sample collection, such as urine, to increase testing among target population. However, with the available technology, this represents a challenge with respect to cost control and logistics in diagnostic laboratories. A major bottleneck associated with NAAT based analysis for *C. trachomatis* diagnostics using urine specimens, is the cumbersome manual procedure to obtain DNA of sufficiently high quality. Automation of the sample preparation is a necessity to reduce both hands-on time and cross contaminations. A method based on magnetic particles for sample preparation, BUGS'n BEADS™, (Genpoint, Norway), has previously been shown to be well suited for isolation of *C. trachomatis* from urine. This automated system currently uses a customized Tecan Miniprep 75 instrument, using about 2.5 hours to process 48 samples. However, laboratories may require even higher throughput, which could be achieved by trying to further reduce the sample preparation time.

Methods: In this study, a simplified magnetic particle based protocol, completing the entire sample processing in only 1.5 hour on the Tecan Miniprep 75 robotic platform, was compared with the original BUGS'n BEADS procedure. A total of 24 urine samples, previously determined positive for *C. trachomatis* using the original BUGS'n BEADS™ method, were analysed using the new preparation protocols in combination with the BDProbeTec detection system for *C. trachomatis*. The amplification control (AC) in the BDProbeTec kit was included for the new method to reveal any potential inhibition.
Results: All 24 samples were positive with the new sample preparation protocol indicating that sensitivity is not reduced in comparison with the original BUGS'n BEADS™ procedure. Moreover, no inhibition was encountered for any of the urine samples as demonstrated by the AC MOTA values, which were all above the cut-off value as defined for the BDProbeTec detection kit.
Conclusion: This new and faster DNA preparation method allows for significant increase in throughput compared to the original BUGS'n BEADS solution. Finally, as a consequence to a reduction in processing time, this new method will allow more laboratories to access full NAAT automation by the introduction of smaller and cheaper robotic systems.

P956

A comparative study of three different PCR assays for detection of *Mycoplasma genitalium* in genital specimens from men and women

A. Edberg, E. Johansson, E. Wikander, A. Höög, T. Ahlqvist, M. Jurstrand, L. Falk, J.S. Jensen, H. Fredlund *(Karlstad, Örebro, SE; Copenhagen, DK)*

Objective: The aims of this study was to compare conventional 16S rRNA PCR, real-time 16S rRNA PCR and real time MgPa PCR as detection methods for *Mycoplasma genitalium* infection. We also wanted to determine the prevalence of *M. genitalium* in patients, both men and women, visiting a STI clinic in a rural area in the west of Sweden.
Methods: First void urine (FVU) and/or urethral swab were collected from 381 men and from 298 women, FVU and/or cervical swab and/or urethral swab were collected. Two hundred and fifteen specimens were used in the PCR comparative study, 100 consecutive specimens, and 36 consecutive specimens from patients with symptoms of urethritis and 79 specimens from patients positive for *M. genitalium* by real time MgPa PCR in the prevalence study. True-positive samples were defined as a specimen positive in any two PCR assays.
Results: The prevalence of *M. genitalium* infection in men and women were 27/381 (7.1%) and 23/298 (7.7%), respectively. In the PCR comparative study, *M. genitalium* DNA were detected in 59/62 (95.2 %) by conventional 16S rRNA PCR, 52/62 (83.9 %) by real time 16S rRNA PCR and in 62/62 (100 %) by real time MgPa PCR of true-positive specimens. *M. genitalium* DNA were detected in an additional 12 specimens with real time MgPa PCR. Four of these specimens were from women with another true-positive sample. An additional 2 specimens, also from women with another true-positive sample, were detected with conventional 16S rRNA PCR.
Conclusion: Real time MgPa PCR indicates slightly higher sensitivity compared to conventional 16S rRNA PCR and increased sensitivity compared to real time 16S rRNA PCR for detection of *M. genitalium* DNA. The prevalence of 7.1 % in men and 7.7 % in women attending a STI clinic is in concordance with other Swedish studies.

P957

A new real-time quantitative TaqMan PCR to detect *Chlamydia trachomatis* DNA in urine and in urogenital swabs

K. Jaton, J. Bille, C. André, G. Greub *(Lausanne, CH)*

Objective: *Chlamydia trachomatis* infection is the most frequent bacterial sexually transmitted disease. Urogenital infections are often asymptomatic and, when remaining untreated, they may lead to severe late complications in women, including infertility and extra-uterine pregnancy. Screening for *Chlamydia trachomatis* infections may be performed on urines and urogenital swabs using molecular detection techniques. However, commercially available methods remain expensive and are associated with large laboratory workload. To reduce costs and increase throughput, we developed a *C. trachomatis* specific real-time quantitative TaqMan PCR (q-PCR).
Material and methods: A q-PCR that may be used on ABI Prism 7900R Sequence System (Applied Biosystem) was developed. This system was coupled to a liquid handling unit (TecanR), allowing automatic pipetting and use of 384 wells micro plates. DNA was extracted with the Magna-PureR (Roche), and q-PCR was performed using 5 μl DNA and 15 μl PCR mixture. Primers and probe were selected from highly conserved sequence of the cryptic plasmid. This new q-PCR was compared to the commercial Cobas AmplicorR (C-PCR) on a total of 646 specimens taken from 564 patients (297 urogenital swabs and 349 urine samples).
Results: The q-PCR exhibited the same analytical sensitivity as C-PCR. The analytical specificity of q-PCR was excellent with no signal detected in presence of bacterial DNA of 7 others *Chlamydia* species and of 15 other bacterial species potentially present in urogenital samples. No inhibition of PCR reaction was observed with q-PCR whereas 19 out of 646 samples (2.9%) were inhibited with the C-PCR. Comparison of the remaining 627 specimens showed an overall agreement of 99.7% between C-PCR and q-PCR (95 +/+, 530 -/-, 2 ±, 0 ±). Considering the C-PCR as gold standard, the specificity was 100% (530/530) and the sensitivity of 98% (95/97). The 2 discrepant results were re-investigated in a second run of C-PCR and q-PCR. One was found strongly positive with the 2 methods. The other was found negative in a second run of Cobas and once positive by q-PCR when 5 additional replicates were done, suggesting that there is a very low amount of DNA in this sample.
Conclusion: The q-PCR is a specific method that exhibits a similar sensitivity than C-PCR for the detection of *C. trachomatis.* Given its high throughput potential and the low costs of q-PCR reagents (about 2 $ per sample), it may contribute to future large-screening program.

P958

First void urine can be used as transport medium for cervical swab specimen for detection of *Mycoplasma genitalium* by polymerase chain reaction

T. Ahlqvist, A. Edberg, E. Johansson, H. Fredlund, F. Aronsson *(Karlstad, Örebro, SE)*

Objective: The objective of this study was to determine if the patients' first void urine (FVU) could be used as transport medium for cervical swab specimen for detection of *Mycoplasma genitalium* infection.
Methods: Cervical swabs and FVUs were collected from 329 women attending a STI clinic during the period of August 2004

to June 2005. Three types of samples were collected from each patient, FVU, cervical swab transported in 2SP medium and cervical swab transported in FVU. All samples were analysed for *M. genitalium* DNA by real time MgPa PCR on SmartCyclerII (Cepheid, Sunnyvale, USA).
Results: *M. genitalium* DNA was detected in 19 of 20 (95%) samples was the cervical swab was transported in the patients FVU. Whereas, *M. genitalium* DNA was detected in 17 of 20 (85%) in FVU samples and 12 of 20 (60%) in cervical swab transported in 2SP medium. When comparing two different DNA extraction methods, manually extraction using Chelex slurry (BioRad, Richmond, USA) and automated biorobot M48 extraction (Qiagen, Hilden, Germany), *M. genitalium* DNA was detected in additional 5 samples (total 25) using the M48 extraction compared to 20 *M. genitalium* positive samples detected using Chelex extraction. Further, inhibition was detected in 6% of the Chelex extracted samples whereas no inhibition was detected using M48 extraction. In a small subset of samples we were able to detect *M. genitalium* DNA after 4 weeks of storage in room temperature.
Conclusion: This study shows that cervical swabs can be transported in the patients FVU were *M. genitalium* DNA is detected by real time PCR. These results indicate that 2SP medium can be excluded from the *M. genitalium* analysis procedure and lead to a reduction of sample numbers from two to one per patient, which will reduce the costs for the analysis.

P959

Automation for generic nucleic acid sample prep in a routine diagnostic laboratory

T.E. Schutzbank, N. Kahmann, C. Jarvis, M. Weimer, A. Yount, K. Lopez *(Dayton, US)*

Objectives: To validate and integrate automated magnetic-particle sample purification systems into the routine workflow of a commercial diagnostic laboratory and to increase the standardization of nucleic acid purification using mid-to-low throughput robotic workstations.
Methods: Nucleic acids were purified using the QIAGEN® BioRobot® EZ1 and M48 workstations using protocols from the EZ1 DNA Bacteria Card and EZ1 Virus Card, and protocols on Infectious Disease Application Package for the BioRobot M48. Bacterial DNA was extracted using the BioRobot EZ1 workstation together with the EZ1 DNA Tissue Kit. BioRobot M48 workstations were used to purify bacterial DNA in combination with the MagAttract DNA Mini M48 Kit and protocols on the Infectious Disease Application Package CD. Viral DNA and/or RNA was purified using the EZ1 Virus Mini Kit on the EZ1 and the MagAttract Virus Mini M48 Kit. Pathogen nucleic acids were extracted from a range of clinical specimen types, including plasma, respiratory samples, swabs, and whole blood. Both artus™ assays and a range of in-house designed downstream assays were used to compare extraction efficiency.
Results: Automated extraction, followed by detection of bacterial DNA from group A and B *Streptococci*, *S. aureus*, and *B. pertussis* showed high sensitivity and specificity while significantly reducing both the mean time-to-result and workflow efficiency compared to manual extraction methods. Cytomegalovirus (CMV) DNA nucleic acids performed well, showing a large dynamic range and allowing accurate quantification using both an in-house designed and a commercially available, artus™, assay. Human Papilloma virus (HPV) DNA and Hepatitis C virus RNA performed well in other commercial assays.
Conclusions: The BioRobot M48 and EZ1 provide standardized and easy-to-use protocols for nucleic acid purification from a wide range of clinical sample types. The BioRobot M48 provides processing capacity (6–48 samples/run) to enable routine applications for mid to high throughput clinical laboratories. The low-throughput of the BioRobot EZ1 (1–6 samples/run) ensures that priority samples can be handled safely and quickly without decreasing laboratory efficiency. Performance of purified nucleic acids in artus™ assays was excellent, illustrating the combined efficacy of BioRobot sample preparation and the artus assays used for diagnostic analyses.

P960

Multiplex real-time PCR for detection of *Candida* infections in blood

Å. Innings, M. Ullberg, A. Johansson, C.J. Rubin, N. Noréus, M. Isaksson, B. Herrmann *(Uppsala, SE)*

Objectives: To develop a multiplex real-time PCR method for detection of the seven most common *Candida* species-causing septicemia: *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. To evaluate a DNA extraction method and detection limits of the PCR for direct detection of *Candida* infections from human venous blood.
Method: A multiplex four channel real-time PCR assay was developed with the target gene RPR1, coding for the RNA subunit of ribonuclease P. The RPR1 sequences of the seven *Candida* species were determined. The multiplex PCR contained four primers and four TaqMan probes labelled with fluorophores of different wavelengths. Three of the probes enabled specific detection of *C. albicans*, *C. glabrata* and *C. krusei* and the fourth allowed general detection of the remaining species. *Candida* DNA was extracted from 3 ml human blood using lyticase and the QIAamp tissue kit.
Results: The multiplex PCR was able to detect 1–10 genome copies when the detection limit was tested repeatedly for the four species *C. albicans*, *C. glabrata*, *C. krusei* and *C. guillermondi*. No significant difference in detection limit was seen when the multiplex format was compared with single species PCR, i.e. two primers and one probe. When the *Candida* DNA was extracted from spiked human blood a detection limit of < 10 cells per ml was reached. The multiplex system has an inbuilt specificity control as only one channel should be positive for a specific species and this was achieved in all runs performed. The sequence data from the seven *Candida* species showed surprisingly large sequence variation. With broad-range primers *C. glabrata* produced an about five times longer amplicon compared to the other species and the nucleotide sequence similarity between *C. krusei* and *C. albicans* were as low as 55%. General primers for satisfactory real-time PCR could not be designed for the species *C. glabrata* and *C. krusei* resulting in the multiplex four-primer system.
Conclusions: A sensitive and specific multiplex real-time PCR was developed for the detection of seven *Candida* species and the identification of *C. albicans*, *C. glabrata* and *C. krusei*. It can be used for detection of *Candida* spp in blood samples.

P961

The application of MALDI-TOF mass spectrometry for the quality control of NCTC strains

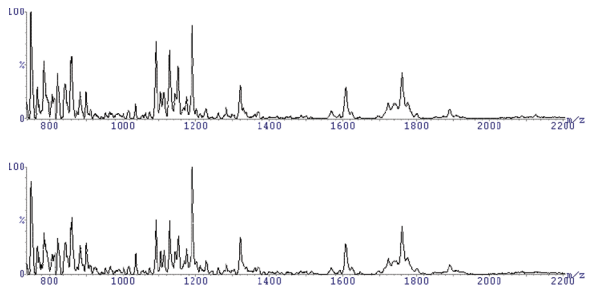
L. Molenaar, D. Dare, H. Sutton, J. Russell, H. Shah, M. Roberts, M. Lunt *(London, Manchester, UK)*

Objectives: Type and reference strains from the National Collection of Type Cultures (NCTC) are used for quality

assessment in both national and international laboratories. The Centre of Infections currently supplies these preserved strains as freeze-dried cultures. The preservation and provision of such cultures in LENTICULE format rather than the freeze-dried cultures is however preferred, since it facilitates easier processing of cultures, increases the distribution rate, provides a more cost effective format and facilitates postage worldwide. To assess the effect of this change in format and thereby ensure that the quality of the NCTC strains remains unchanged, MALDI-TOF mass spectrometry is used for direct comparison of the respective NCTC preserved strains.

Method: Ten NCTC type and reference strains, representing 6 genera and 9 species, were simultaneously revived from freeze-dried and from the equivalent LENTICULE discs that were preserved between 1996 and 2004. The intact microbial cells from equivalent samples were inoculated onto the same MALDI target plate and co-crystallised with the same batch of MALDI matrix. Laser interrogation of the surface associated molecules from the paired type and reference strains provided the comparative MALDI-TOF mass spectral profiles used for assessing the differences associated with each preservation format.

Results: Mathematical interrogation of the mass spectral profiles demonstrated there is negligible difference between each of the organisms recovered from the two preservation methods. Surface associated molecules of intact microbial cells therefore remain unaltered after preservation by lenticulation.



Comparative spectrum of Pseudomonas aeruginosa 10662 cultured on CLED agar over 500 to 2200 mass range, demonstrating the similarity between the preservation methods. The top sepctrum represents the strain revived from LENTICULE disc and the bottom sepctrum represents the same strain revived from freeze-dried.

Conclusion: The application of MALDI-TOF mass spectrometry for the quality control of NCTC strains suggests that LENTICULE production has not resulted in altered morphotype and ensures the quality of the NCTC strains is maintained.

P962

LightCycler® SeptiFast Test: rapid detection of nosocomial pathogens by real-time PCR

T. Emrich, M. Moczko, S. Lohmann, J. Mayr, H. Stockinger, G. Haberhausen *(Penzberg, DE)*

Objective: At present, one of the main medical problems in hospitals is the increasing number of nosocomial (hospital-acquired) infections. Epidemiological data show that a limited number of microorganisms are responsible for the majority of bloodstream infections. Twenty-five species account for more than 90% of all nosocomial pathogens. A rapid diagnosis of Sepsis and the correct identification of the causative pathogen followed by immediate and appropriate antimicrobial therapy is the key of success in the management of bacteraemia and fungaemia in septic patients, but not limited to these.

Methods: The LightCycler® SeptiFast Test was developed to detect and differentiate up to 25 pathogenic microbial DNA(s) in human whole blood. Specimen preparation is based on a semi automated procedure using the MagNa Lyser followed by a manual spin column based nucleic acid preparation. Amplification and detection are automated using the LightCycler 2.0 Instrument.

Results: Compared to classical methods (blood culture followed by gram staining and species identification based on culture methods), the LightCycler® SeptiFast Test offers an improved sensitivity and a much better time to result. In most cases, the result will be available in about 4.5 hours instead of two to three days. In case of a positive PCR result, therapy can be adjusted about two days earlier, which might lead to significant savings for the hospital as well as to improved outcome for the patient. In case of negative PCR results, blood culture remains the basis for medical decisions.

Conclusion: The LightCycler® SeptiFast Test is the first IVD assay using PCR for the rapid detection and identification of bacterial and fungal pathogens involved in nosocomial infections directly from whole blood. Here we present non-clinical performance data as well as selected data from several external clinical studies to illustrate the ability and power of the assay.

P963

Comparison of real-time and conventional PCR assays for diagnosis of Cat Scratch Disease in patients with lymph node enlargement

C. Le Brun, S.J. De Martino, Y. Hansmann, J. Debs, C. Walter, Y. Piemont, B. Jaulhac *(Strasbourg, FR)*

Objectives: Real-time polymerase chain reaction (PCR) is a novel rapid method for the detection and identification of fastidious growing bacteria like *Bartonella*. The aims of this study were (i) to develop a real-time PCR based method able to differentiate the main *Bartonella* species involved in human endocarditis and in Cat Scratch Disease (CSD) (ii) to compare real-time PCR with conventional PCR for diagnosis of CSD.

Methods: A real-time PCR assay, using a double hybridization probe format (Light Cycler) was developed. Identification and differentiation of *Bartonella* species was based on the amplification by real time PCR of a 205-bp fragment of the *glt*A gene followed by Tm determination. Conventional PCR targeted a 414-bp fragment of the *htr*A gene of *B. henselae* (i). We evaluated 113 samples (lymph node biopsy or cytopunctures) over a ten-year period. The patients were divided into three groups according to the number of positive diagnostic criteria for CSD: 23 patients with definite CSD (two or more classical criteria), 69 with possible CSD and 21 for whom another diagnosis was retained used as a control group.

Results: Conventional PCR detected *B. henselae* only, whereas the real-time PCR method could detect and differentiate *B. henselae*, *B. quintana*, *B. koehlerae*, *B. vinsonii*, *B. alsatica* and *B. berkhoffii*. Specificity was 100% for the both assays. Sensitivity was 78% by both conventional and real-time PCR assays on samples from patients with definite CSD. Real-time PCR coupled to automate DNA extraction on the MagNA Pure robot (Roche) was less susceptible to inhibitors than manual extraction (6 vs.0 by MagNA Pure).

Conclusion: This Light Cycler PCR assay was sensitive, specific, and less time consuming than conventional PCR. Therefore, real-time PCR analysis could be used for CSD diagnosis in combination with epidemiological, serological and histological criteria. (1) Hansmann Y, De Martino S, Piémont Y, Meyer N, Mariet P, Heller R, Christmann D, Jaulhac B. Diagnosis of Cat Scratch Disease with detection of *Bartonella henselae* by PCR: a study of patients with lymph node enlargement. J Clin Microbiol 2005; 43: 3800–3806.

Fast detection of respiratory microbes

P964

Advances in PCR detection of *Pneumocystis jiroveci* in immunocompromised patients: count seems to be important

J. Lochmanova, Z. Racil, M. Lengerova, D. Minarikova, P. Volfova, D. Dvorakova, J. Mayer *(Brno, CZ)*

Objectives: *Pneumocystis jiroveci* remains one of the important causes of pneumonia in immunocompromised patients. Detection of this fungus in bronchoalveolar lavage (BAL) fluid using nested PCR has great sensitivity and specificity for diagnostics of *Pneumocystis* pneumonia (PCP). Because this two-step method is rather time consuming we decided to replace it by TaqMan real-time quantitative PCR targeting the neighbouring region of the same gene (mtLSU rRNA gene).
Methods: Between VI/99 and X/2005, a total number of 266 BAL specimens of immunocompromised patients were tested using nested PCR assay. Forty-nine specimens (18,4%) were *P. jiroveci* positive. Twenty-nine *P. jiroveci* positive and 10 negative specimens were retested using novel real-time PCR assay. Results of both methods were compared and detected copy numbers were correlated to clinical findings.
Results: There was absolute concordance between positive and negative results obtained by both methods. The real-time PCR assay is able to detect< 10 copies of a plasmid standard per tube and it has the same sensitivity as the nested PCR. The copy numbers of the target gene in positive samples varied between 6 and 139534 copies per tube; median was 121 copies per tube. The 29 positive patients that were retrospectively clinically evaluated could be divided into 3 groups: (i) 15 (51.7%) patients with PCP had median copy number of 4766 (range 12 – 139534) copies per tube, (ii) 6 (20.7%) patients with polymicrobial pneumonia (*P. jiroveci* + another pathogen) had median copy number of 334 (range 13 – 11237) copies per tube and iii) 8 (27.6%) patients without PCP had median copy number of 70 (range 6 – 138) copies per tube.
Conclusions: We developed a rapid and sensitive real-time PCR assay for detection of *P. jiroveci*. Using this assay, we are able to obtain results in about 4 hours from sample delivery, contrary to 6 hours that are necessary for nested PCR. Moreover this assay seems to discriminate patients with PCP and *P. jiroveci* carriers. Certainly, more samples must be tested to determine the cut-off values more precisely. This work was supported by Ministry of Health of the Czech Republic (grant no. NR/8452-3).

P965

Evaluation of a new assay for the detection and differentiation of *B. pertussis, B. parapertussis* and *B. bronchiseptica* based on real-time PCR technology

A. Burmeister, T. Grewing, H. Petersen, N. Kahmann, T.E. Schutzbank, M. Hess *(Hamburg, DE; Moraine, US)*

Objective: Laboratory diagnosis of whooping cough, is not well standardized. Different PCR systems have been developed for the detection of *Bordetella pertussis*, mainly based on insertion sequences (IS) as target region. IS show copy numbers of up to 250 per cell, which makes these systems highly sensitive? However, IS are transposable DNA elements, capable of horizontal transfer across species and of spontaneous elimination. These are characteristics that tend to compromise specificity of PCR based diagnostic systems. The goal of this work was to develop and to evaluate a highly sensitive, absolute specific and reliable IVD assay, based on Real-Time PCR technology for the detection and differentiation of the human pathogenic *Bordetella* species *B. pertussis*, *B. parapertussis* and *B. bronchiseptica*.
Methods: We developed an IS-independent PCR system for the simultaneous detection of *B. pertussis*, *B. paraperussis* and *B. bronchiseptica* and the differentiation of these species by melting curve analysis using the LightCycler Instrument (Roche Diagnostics). We combined this system with an IS-dependent (IS481/IS1001) PCR system and an Internal Control (IC) PCR. Testing type strains of all known Bordetella species, different clinical isolates of human pathogenic Bordetella species and about 50 different bacterial, viral and fungal isolates validated specificity. The analytical and clinical sensitivity of the assay was evaluated by Probit analysis and by testing over 200 clinical specimens by different laboratories.
Results: We were able to develop a one tube Real-Time PCR assay for the detection and differentiation of *B. pertussis*, *B. parapertussis* and *B. bronchiseptica*. An Internal Control was also implemented into the system. Testing DNA preparations of all known Bordetella species and of viral, bacterial and fungal isolates, either closely related to Bordetella or causing similar symptoms observed no cross-reactivity. The analytical sensitivity of the assay for *B. pertussis* determined by Probit analysis is 1.3 copies/μl ($p \leq 0.05$). Clinical sensitivity and specificity was demonstrated by testing over 200 clinical specimens.
Conclusion: We could demonstrate that our new Real-Time PCR assay for the detection and differentiation of human pathogenic *Bordetella* species combines the high sensitivity of Insertion Sequence based PCR systems with the reliable specificity of a IS independent PCR system and the security of an Internal Control in a single reaction.

P966

Molecular diagnosis of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infections by real-time NASBA

P. van Aarle, P. van Deursen, K. Loens, P. Sillekens, M. Overdijk, M. Ieven, H. Goossens *(Boxtel, NL; Edegem, BE)*

Objectives: Evaluation of a nucleic acid amplification procedure for the detection of *Mycoplasma pneumonia* and *Chlamydia pneumoniae* in respiratory tract specimens.
Methods: Using RNA as the principal target, amplification was performed with NASBA reagents from the NucliSens Basic Kit and in-house produced primers and molecular beacon probes. Internal Control (IC) RNAs were developed to monitor the procedures at the individual sample level. For nucleic acid extraction from respiratory samples, the NucliSens miniMAG method based on magnetic silica was used. Methods were evaluated on sputa, bronchus aspirates, bronchoalveolar lavages and throat swab samples from patients with CAP.
Results: For respiratory samples, optimal IC RNA input levels were established as a compromise between reliable detection of the IC RNA and minimal effect on the sensitivity for the detection of pathogen RNA. Both IC RNAs were spiked into the samples and nucleic acid extraction was followed by amplification in duplicate with either of the primer/beacon combinations. Sensitivity, defined as the 90% hit rate, was found

ISSN: 1470-9465

to be 60 copies of RNA per extraction for *Chlamydia pneumoniae* and 130 copies for *Mycoplasma pneumoniae*. Successful nucleic acid extraction was reflected by efficient IC RNA amplification. Only incidentally, invalid results were obtained, most of which could be resolved by a second amplification analysis of the nucleic acid extract. A total of 15 samples was found positive (9 for *Mycoplasma pneumoniae* and 6 for *Chlamydia pneumoniae*), 14 of which could be confirmed by a second molecular method.
Conclusions: Real-time NASBA amplification in potential is a valuable tool for the molecular diagnosis of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. Multiple amplification analyses, each with its own IC RNA, can be performed on a single nucleic acid extract. Further evaluation is needed to confirm the clinical value of these methods.

P967

Development of a real-time PCR using SYBR Green and melting curve analysis to identify *Bordetella pertussis* and *Bordetella parapertussis* in clinical specimens, and evaluation of its effectiveness in current practice

P. Lehours, A. Menard, J. Sarlangue, C. Laguente, C.H. Bebear, F. Megraud, B. De Barbeyrac *(Bordeaux, FR)*

Objective: Whooping cough affects now young adults and teenagers, thus being able to contaminate infants unvaccinated or incompletely vaccinated. In this population, it evolves readily into atypical or severe forms difficult to diagnose and a biological confirmation is necessary. Thus molecular techniques represent invaluable tools. Here, we describe a real-time PCR assay for the identification of *B. pertussis* and *B. parapertussis* in clinical specimens
Methods: The available IS481 sequences of *B. pertussis* were aligned to identify conserved regions for the primer design. Primers for the detection of *B. parapertussis* were designed on the IS1001 sequence. A universal PCR targeting the 16S rDNA gene was also developed to be used as a control for extraction and for absence of PCR inhibitors. The PCR primers sets were tested on DNA extracted from the *Bordetella* species, and from diverse bacterial species commonly found in humans. The SYBR Green® real-time PCR amplification and melting curve (Tm) analysis were carried out in an ABI PRISM® 7000 thermocycler (Applied Biosystems), and followed by a melting program. A retrospective study was performed on positive (n = 19) and negative (n = 13) nasopharyngeal aspirates (NPA). We tested prospectively 131 NPA received between April 2004 and November 2005.
Results: When using the control strains, only DNA extracted from *B. pertussis* and *B. parapertussis* led to amplification, demonstrating the specificity of these two PCRs. Melting curve analysis of the amplicons from *B. pertussis* and *B. parapertussis* control strains exhibited Tm of approximately 79°C and 86°C, respectively. Serial dilutions of the extracted DNA from bacterial suspensions were tested using this assay and the regression curve was linear from 105 to 1 bacterial genome with slopes closed to that of the maximum efficiency. For the retrospective analysis, the 19 positive samples were all positive for *B. pertussis*, and no peak appeared for the 13 negative samples. Prospectively, this assay allowed the detection of 21 positive clinical samples (18 *B. pertussis*, 3 *B. parapertussis*), among these 14 could not have been isolated by culture.
Conclusion: We have developed a new real-time PCR for the identification of *B. pertussis* and *B. parapertussis* targeting the IS sequences. This method, which uses the SYBR green chemistry and analysis of the melting point, is rapid, sensitive and specific, and can be easily implemented in a setting where a real time PCR apparatus is available.

P968

Diagnosis of pertussis by real-time LightCycler PCR

M. Alves, G. Rocha, A. Magalhães-Sant'Ana, C. Nogueira *(Coimbra, PT)*

Objectives: Pertussis (whooping cough) is a well-known childhood respiratory disease and is caused primarily by *Bordetella pertussis* infection. Although immunization programs brought the disease under control for decades, studies conducted in recent years indicate that is re-emerging as a potential threat even among those who are vaccinated. The disease is now quite prevalent among adults and adolescents in whom the disease symptoms are often atypical. They are a source of *B. pertussis*, and this is a major reason why this disease is being transmitted to children. Definitive diagnosis of pertussis has traditionally been made by culture. However it is insensitive and slow. Serological tests are more sensitive than culture, but provide late or retrospective diagnosis. Other commercial immunoassays do not distinguish between *B. pertussis* and *B. parapertussis* infection. Our aim was to perform a rapid highly specific and sensitive detection of *B. pertussis* and *B. parapertussis* that is essential for providing appropriate therapeutic intervention and controlling transmission of the disease.
Methods: Between December 2004 and November 2005 a total of 126 nasopharyngeal aspirates (NPA) were analysed for *B. pertussis* detection and 15 of those were also analysed for *B. parapertussis*. In 115 the patients were aged 1 day to 18 month (59 female and 56 male) with a mean of 3.34 months. 3 NPA were obtained from 3 older children (3 female), median age 11.33 years and 7 NPA from household contacts.Nucleic acid was extracted from NPA with the QIAamp DNA Mini Kit according to manufacturer's instructions. Extracted DNA was directly analysed by LightCycler ® PCR without storage. Primers and probes from IS481 and IS1001 for *B. pertussis* and *B. parapertussis*, respectively, were adopted from the work of Kösters K. et al., 2002, J Clin Microbiol: 1719-1722.
Results: From the 126 NPA analysed, 52 (49 children and 3 adults; 28 female and 24 male) were positive for *B. pertussis*. For *B. parapertussis*, all samples tested were negative.
Conclusions: The standard LightCycler protocol used can complete an analysis of up to 30 samples plus negative and positive controls within a total assay time of 55 minutes. It achieves high specificity and sensitivity by adding specific probes. In sum, real-time LightCycler PCR is suitable for diagnosis of *B. pertussis* and *B. parapertussis* infections in a routine diagnostic laboratory.

P969

Bordetella pertussis real-time PCR: sample preparation by different kits from one manufacturer

M. Riffelmann, C. Wirsing von Koenig, J. Schmetz, S. Bock *(Krefeld, DE)*

Background and aims: Pertussis is a re-emerging disease, and its diagnosis is increasingly done by real-time PCR. Commercial kits for preparing DNA from respiratory material are not CE marked for this purpose.

Methods: More than 100 nasopharyngeal swabs, tracheal secretions and other respiratory materials were spiked with various amounts of *B. pertussis* cells. Commercially available ion-exchange chromatography column kits from one company (QIAGEN) were compared: (i) the QIAamp DNA mini kit, which is validated for DNA extraction from various materials, (ii) the QIAamp Min Elute Virus Vacuum kit, (iii) the QIAamp Virus BioRobot MDx kit, and (iv) the QIAamp DSP Virus kit, which is CE marked. All kits are validated only for preparation of viral RNA and DNA from cell-free material and from blood. PCR was performed by two real-time PCR's based on a hybridization probes format (LightCycler, Roche) and on a TaqMan format (SDS 7700, Applied Biosystems). Both PCRs amplified different parts of the insertion sequence IS 481 from *B.pertussis*.
Results: All four kits tested could be used for the preparation of *B. pertussis* DNA from respiratory materials. The reproducibility of all kits was comparable. All procedures showed linearity of extraction over a broad range of bacterial DNA (5–500 000 organisms/PCR). The MinElute Virus Vacuum kit and the DSP Virus kit did not effectively remove inhibitory substances from respiratory samples.
Conclusions: Commercial nucleic acid preparation systems were effective in preparing DNA for *B.pertussis* real-time PCR. The DNA Mini Kit could be used for the extraction of single samples, and the Virus BioRobot MDx Kit could be used for preparation of 32–96 samples with the BioRobot MDx instrument. Our findings underline the necessity of validating nucleic acid preparation kits for different clinical materials and applications.

P970

Comparison of specificity and sensitivity of various diagnostic methods of ureaplasma respiratory tract infections in newborns

M. Biernat-Sudolska, D. Rojek-Zakrzewska, B. Zawilinska *(Cracow, PL)*

Objective: Ureaplasmas can cause respiratory insufficiency in newborns. Detection of ureaplasma infections by culturing is difficult and time-consuming. In newborns an additional problem is the necessity of antibiotic administration before the diagnostic procedures are started. Antibiotics inhibit the growth of ureaplasmas limiting reliability of the culture method. The purpose of our study was to estimate various diagnostic methods of urea plasma infections in newborns with respiratory disorders.
Methods: Five hundred newborns were examined. Specimens were cultivated in PPLO medium according to Hayflick (CH) and parallelly examined with a BioMerieux test (BM). The polymerase chain reaction (PCR) assay was also applied to test specific regions of 2 ureaplasma species occurring in man: Ureaplasma urealyticum (Uu) and Ureaplasma parvum (Up).Endotracheal aspirates were collected from each infant and placed in transport medium BM. Next they were sub cultured in liquid and solid PPLO media and in BM medium. DNA was isolated from the transport-culture medium BM after 18–24 hours of incubation. Specimens were centrifuged at 14 000 g and the DNA was denaturated at 950 C for 5 minutes. Two pairs of primers specific for Uu and Up were used. DNA from reference strains was used as a positive control. The PCR products were separated electrophoretically in 2% agarose gel and visualized with ethidium bromide under UV.
Results: Ureaplasmas were detected in respiratory tracts of 79 (16%) newborns examined. CH showed positive results in 68 cases, BM in 77 cases and PCR in 75 cases. Correlation of results of CH with BM, CH with PCR and BM with PCR was 97%, 89% and 90%, respectively. Sensitivity and specificity of PCR in comparison with CH was 86% and 98%, respectively and BM - 96% and 98 %. In 40 cases the other bacteria made it impossible to recognise the results of ureaplasmas in culture. PCR results were then definitive. In 4 cases the positive result of BM test could not be confirmed by other methods. Only in 1 case the positive result of PCR was not confirmed by culture result.
Conclusion: PCR, as a highly sensitive and specific method, can be recommended in rapid diagnostics for respiratory infections in newborns suffering from respiratory disorders. It allows detecting ureaplasmas in case of concomitant infections and identifying their species.

P971

Evaluation of real-time nucleic acid sequence-based amplification and dipstick-based detection with conventional NASBA and PCR for rapid detection of *Mycoplasma pneumoniae* in respiratory specimens

K. Ohkusu, M. Komatsu, N. Ishiwada, T. Hoshino, N. Takeda, H. Sakata, T. Takeshima, T. Ezaki *(Gifu, Nara, Chiba, Hokkaido, Shizuoka, JP)*

Objectives: *Mycoplasma pneumoniae* is an important etiologic agent of respiratory tract infections and is responsible for 10 to 20% of community-acquired pneumonia in adolescents and adults. *M. pneumoniae* is not sensitive to β-lactam antibiotics, which are often used for the empiric treatment of lower respiratory tract infections (LRTI). Therefore, rapid and accurate diagnostic methods are likely to improve the management of patients with *M. pneumoniae* infections. In this study, we evaluated a real-time NASBA assay for *M. pneumoniae* RNA in clinical specimens and compared it with real-time PCR. In addition, we developed a nylon membrane strip method for the rapid visual detection of nucleic acid hybridization.
Methods: Respiratory specimens from 140 paediatric and adult patients with acute LRTI at five hospitals in Japan were included in this study. Nucleic acids were extracted using NucliSens® magnetic extraction kit (bioMérieux). Real-time NASBA assays were performed using NucliSens® basic kit and EasyQ analyser (bioMérieux) with primers OT2156NBK, OT2157 and a molecular beacon probe (Loens *et al.*, J Clin Micobiol 2003, 41:4448–50). For a reference method, real-time PCR using SYBR green I was performed on an iCycler iQ system (Bio-Rad) as described by Morozumi *et al.* (J Infect Chemother 2004, 10:274–9). Detection of NASBA amplified products on a nylon membrane strip was achieved by sandwich nucleic hybridization with two target-specific oligonucleotide probes, one for capture and the other for detection. The formation of sandwich complex, within 15 minutes, on the membrane strip resulted in a visually apparent aggregation of nucleic acid target and latex conjugated probes at the capture zone.
Results: For both NASBA and PCR assays, 12 of the 140 specimens tested were positive and none of 128 PCR-negative clinical specimens were positive by real-time NASBA. The analytic sensitivity of the strip detection was 200–1000 copies of wild type *in vitro*-synthesized RNA generated by a 60 minutes NASBA amplification assay. All 12 positive specimens were positive by the visual strip detection in combination with NASBA.
Conclusions: The sensitivity and specificity of the real-time NASBA were 100% using a real-time PCR assay as the standard. Furthermore, a combination of the NASBA assay and strip detection could facilitate the use of NASBA in resource-limited settings and in point-of-care testing.

P972

Identification of *Legionella* species using DNA sequence analysis and online tools

N.K. Fry, B. Afshar, W. Bellamy, A. Underwood, R.M. Ratcliff, T.G. Harrison *(London, UK; Adelaide, AU)*

Objectives: Fifty species of *Legionella* have now been described, and the identification of the majority of these by classical phenotypic methods is increasingly difficult. The aim of this study was to investigate the feasibility of providing a dedicated database allowing the on-line identification of putative *Legionella* spp. following standard PCR amplification and sequencing using a previously published identification scheme for *Legionella.* The *Legionella* mip gene sequence database is created by the Health Protection Agency, Centre for Infections (http://www.hpa.org.uk/infections/default.htm) and can also be reached via the EWGLI website (www.ewgli.org).
Methods: DNA sequence can be entered as text or by uploading sequencing chromatogram files. The database provides the following functions: (i) an alignment of all the sequences from the reference alignment, top five database matches and the user sequence, (ii) a neighbour-joining tree of the alignment, including the reference species, five closest matches from the database and the user sequence, and (iii) an alignment of the eight sequences from the combined alignment that are most similar to the user sequence.
Results: The ability of centres to correctly identify *Legionella* species was assessed following the distribution of two proficiency panels each comprising 10 coded isolates. Most centres, 7 of 10, and 11 of 13 correctly identified all isolates tested. Furthermore training needs were highlighted and addressed resulting in improvement in sequence quality.
Conclusions: Genotypic identification of *Legionella* species is essential for reference laboratories. Standard protocols, dedicated identification libraries, and online tools provide a valuable resource to help achieve this goal. It is anticipated that additional genes including those coding for: 16S rRNA, *Rpo*B, *Rnp*B, and *Gro*EL, will be added to the current identification system to aid in the characterization and identification of known and potential novel members of this genus.

P973

A real-time PCR for *Mycoplasma pneumoniae*

V.J. Chalker, D. Pitcher, T.G. Harrison *(London, UK)*

Objectives: To create an improved real-time PCR for *Mycoplasma pneumoniae* detection.
Methods: Using primers directed to the P1 adhesion gene a real-time PCR was developed with FRET probes to simultaneously detect *Mycoplasma pneumoniae* and an inhibition control. Sensitivity was ascertained using samples previously confirmed by culture and/or serology and specificity using a panel of respiratory bacteria and *Mollicute* species. Since introduction into routine practice 45 clinical samples have been screened by this method.
Results: The assay was used on a panel of 175 respiratory samples from patients with pneumonia for which data regarding other respiratory pathogens was available. The specificity was excellent, 96.6%, with no cross-reactions with other *Mollicutes* or respiratory bacteria. The sensitivity was calculated as 60% when compared to serology and the LDL of the assay was 5 x 102 organisms m/l, 10 fold lower than previously described methods. Inhibition was detected in 24% of neat sample extracts and reduced to 4% in samples diluted by a factor of 10. Since introduction a total of 4/45 (8.9%) clinical samples tested were positive, 1 lung biopsy, 1/4 sputa (25%) and 2/4 (50%) NPA.
Discussion: A highly specific rapid method of real-time PCR for *Mycoplasma pneumoniae* detection is described with greater sensitivity than previous assays.

P974

Detection of *Legionella* DNA in clinical samples using real-time PCR

L. Franzin, D. Cabodi, N. Bonfrate, F. Tortorelli *(Turin, IT)*

Objectives: Culture is the gold standard for the diagnosis of *Legionella* infection. However several days are required to obtain a positive result. DNA techniques are promising for rapid detection. In this study a real time (RT)-PCR system was evaluated for the direct detection of *Legionella* from clinical samples and compared to culture.
Methods: Culture and RT-PCR was performed on 78 respiratory samples (10 sputum, 44 bronchoalveolar lavage fluid, 19 bronchial aspirate, 5 lung tissue) from 76 patients with pneumonia. *Legionella* infection (LD) was confirmed in 23 patients by positivity to at least one of these tests: urine antigen detection (ELISA), serology (fourfold rise in antibody titres by indirect immunofluorescence and/or micro agglutination test) and culture. Aliquots of the untreated, heat-treated and acid-washed samples were plated on BCYE, BMPA and MWY. The plates were incubated at 37°C for 15 days. Blood samples from 11 symptomatic old patients involved in an LD outbreak were also examined. RT-PCR for *L.pneumophila* (Lp) and *Legionella* spp (L.spp) was performed after DNA extraction with iCycler and BIO-RAD reagents, according to a modified protocol.
Results: Culture from respiratory samples was positive from 17 patients. *Lp* serogroup 1 was isolated from 15 subjects, *Lp* 3 from one and *L.bozemanii* from another. RT-PCR was positive in a total of 25 (32%) samples. 24/25 (96%) samples from LD patients were positive by RT-PCR, while 52/53 (98%) samples from patients without LD were negative. Discrepancy was observed in only one case (1.3%) for category of patients with and without LD. Three patients with proven LD was positive by RT-PCR for *L.* spp and negative for *Lp*, but one of these was culture positive for *L.bozemanii*. All culture positive respiratory samples were RT-PCR positive, except one where Lp3 was isolated. Concordance for respiratory samples was 97.4%. All blood culture samples were negative, while RT-PCR was positive from 4/6 outbreak patients with laboratory confirmed LD and from 5/5 patients with clinical, but not laboratory confirmed, LD.
Conclusion: Laboratory diagnosis LD is better achieved by combined urine antigen detection, serology and culture. Results of this study showed good concordance between the criterion based on traditional methods and RT-PCR. This new sensitive technique, that needs to be further evaluated, offers the advantage of a rapid diagnosis.

P975

Rapid diagnosis of *Mycobacterium tuberculosis* by a triplex real-time PCR assay

W. Sougakoff, C. Truffot-Pernot, G. Millot, V. Jarlier *(Paris, FR)*

Objectives: Sensitive and rapid techniques to detect and identify *Mycobacterium tuberculosis* directly in clinical specimens are important for the diagnosis and management of patients with tuberculosis. In this study, we report the evaluation of a real-time PCR assay targeting the IS6110

element and the RD9 specific region as a tool to rapidly diagnose and differentiate *M. tuberculosis* and *M. canetti* (IS6110 positive or negative – RD9 positive) from the other mycobacterial species included in the *M. tuberculosis* complex (IS6110 positive – RD9 negative) such as *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *M. caprae* and *M. pinnipedii*.

Methods: The performances of the assay were assessed from 166 clinical samples collected from patients over a two months period. DNA preparations and amplifications were carried out with a new kit developed by BioRad (Bio-Rad Real Time TB Assay), following the manufacturer's instructions. An internal control was added before DNA extraction in order to measure the efficiency of DNA recovery and check for the absence of inhibitors.

Results: The BioRad Real Time TB assay correctly identified 17 specimens that were proven by culture to contain *M.tuberculosis*, of which 16 were positive for IS6110 and RD9 and 1 was positive for RD9 but negative for IS6110. Seven samples IS6110-positive but RD9-negative was shown by culture to contain *M. bovis* ($n = 6$) or *M. africanum* ($n = 1$). Eight samples that remained culture-negative were found to be positive to either IS6110-based amplification ($n = 2$) or RD9-based amplification ($n = 3$) or both ($n = 3$). Finally, 131 of the 134 samples negative for both IS6110 and RD9-based amplifications remained negative on culture, the three culture-positive amplification-negative samples yielding only a few colonies of *M. tuberculosis* on culture.

Conclusions: The assay, based on the real-time amplification of three distinct targets (IS6110, RD9 and Internal Control) was found to be robust, sensitive and specific, and is characterised by a complementary pattern of identification: broad for the IS6110-based amplification and specific of *M.tuberculosis* and *M.canetti* for the RD9-based amplification.

Diagnostic and laboratory methods for bacteria - I

P976

The antimicrobial susceptibility and API CORYNE profile code for 34 strains of the newly reported *Corynebacterium resistens*

Y. Otsuka, Y. Kawamura, Y. Yamaguti, H. Takahashi, K. Ohkusu, H. Hanaki, T. Ezaki *(Tokyo, Gifu, JP)*

Objectives: In 2005, *Corynebacterium resistens* was reported as a new species of *Corynebacterium* demonstrating multi-resistance to antimicrobics (J. Clin. Microbiol. 43:3713–3717). Multi-resistant corynebacteria such as *C. jeikeium* and *C. urealyticum* are well known for contributing to mortality in immunocompromised patients with haematological malignancies. We report on API CORYNE (bioMérieux, France) and minimum inhibitory concentration (MIC) profiles observed for *C. resistens*.

Methods: During a period of 7 years (March 1998 through September 2005), 34 isolates of an unknown Gram positive bacilli were recovered from blood, sputum, operative wound, pus, otorrhea, and CV line specimens. Based on 16S rRNA sequencing of a 1400-bp segment, the organism was identified as a new species of *Corynebacterium* and named *C. resistens*. API CORYNE (bioMérieux, France) examined it according to a designated method. Microdilution antimicrobial susceptibility studies were performed using 1% Tween 80 as a supplement to Mueller-Hinton broth to stimulate the lipiphilic *C. resistens*.

Results: Four API CORYNE patterns were observed: 0100004 (14 strains), 4100004 (13 strains), 6100004 (6 strains), and 2100004 (1 strain). While alkaline phosphatase was positive in all strains, pyrrolidonyl arylamidase and pyrazinamidase varied depending on the strain. While corynebacteria are defined as oxidizing glucose and ribose, this characteristic is dependent on inoculum density; glucose and ribose reactions were negative in API CORYNE. MIC results for β-lactams, aminoglycosides, clindamycin, fluoroquinolones, and tetracycline demonstrated resistance, whereas, results for vancomycin were susceptible. With respect to vancomycin, the MIC 90 was 2.0 μg/ml while linezoloid showed the most activity at 0.5 μg/ml.

Conclusions: *C. resistenss* is capable of causing serious infections comparable to *C. jeikeium*. It is important to assess the clinical significance of each isolate recovered from clinical specimens. Furthermore, there is a need to update the API CORYNE date base to reflect *C. resistens*. Linezoloid or vancomycin should be given consideration as first-line drugs.

P977

Rapid phenotypic detection of pneumococci and beta-haemolytic streptococci directly from blood cultures

J. Montgomery, B. Mayall *(Melbourne, AU)*

Objectives: Lance field groups A, B, C and G are the major beta-haemolytic *streptococci* (BHS) causing human infections. Life threatening toxic shock-like syndromes and bacteraemia are not restricted solely to Group A *Streptococci* and the incidence has recently increased. The aim of this study was to determine the accuracy of pneumococcal antigen test (PN) for detection of *S. pneumoniae* (SPN) and a modified streptococcal grouping (MSG) test, directly from blood culture broth (BCB) on the day of detection of a bacteraemia (Becton Dickinson, USA).

Methods: If Gram-positive cocci resembling *Streptococci* were seen on Gram, 10 ml BCB was centrifuged in a gel tube. Pneumococcal antigen (PN) test was done from the supernatant using Wellcogenä *S. pneumoniae* kit as instructed by the manufacturer (Remel Inc. USA). PN negative BCB were then grouped by the MSG test using the Streptococcal Grouping Kit (Oxoid Ltd, England). The supernatant was discarded; the bacterial pellet resuspended in 0.4ml of extraction enzyme and grouping was performed as instructed by the manufacturer. Results were compared with standard culture methods.

Results: PN was performed on 203 bottles with Gram stain resembling *Streptococci*. A total of 80 were SP. Two false negatives but no false positive reactions occurred. Sensitivity (SN) was 98%; Specificity (SP) and Positive Predictive Value (PPV) were 100% and Negative Predictive Values (NPV) 98%. MSG was then performed on 115 PN negative bottles from 110 patients. Five *Enterococci* auto-agglutinated and were excluded; 77 were groups A- D, F or G and 36 non-groupable *Streptococci*. There were three false positive and twelve that did not group by this method. Group D was problematic with six negative *Enterococci* and four negative *S. bovis*. Overall SN, SP, PPV and NPV were 87, 90, 95 and 75% respectively. Of 36 BHS: Group A (13), B (8), C (7) and G (8) 34 grouped correctly with SN 95%, SP 99%, PPV 97% and NPV 98%.

Conclusion: Direct grouping of blood culture with a Gram resembling *Streptococci* but PN negative, was unreliable for *Enterococci*, but gave accurate and rapid phenotypic detection of Groups A, B, C and G. This has significant impact on the laboratory turnaround time, may impact on therapy and the clinical relevance of results especially in severe invasive disease.

P978

Perioperative monitoring of the endotoxin chemical markers and procalcitonin blood plasma concentrations in cardiosurgical patients

N.V. Beloborodova, D.A. Popov, A.Y. Olenin, A.S. Archipova, E.A. Chernevskaya *(Moscow, RU)*

Objectives: 3-hydroxy fatty acids (3-OH FAs) of 10–18-carbon chain lengths are constituents of the lipopolysaccharide of Gram-negative bacteria. Thus, these acids can be used as chemical markers for determining endotoxin. The aim was to study the perioperative blood plasma levels of 3-OH FAs and marker of systemic inflammation of bacterial origin - procalcitonin (PCT) - in cardio surgical patients (pts.).
Methods: Blood plasma samples from 22 consecutive adult pts. with acquired heart diseases (mean age 49 (42–56) years) were taken before and on the 1st day after surgery. PCT concentrations were measured by immunoluminometric method (PCT LIA, B.R.A.H.M.S Aktiengesellschaft GmbH, Germany). The levels of 3-hydroxytetradecanoic acid (3h14) were detected by gas chromatography-mass spectrometry (GC-MS) (HP-5973, Agilent technologies, USA). Postoperatively all pts. were divided into two groups: with less than 2 ng/ml (Group A, $n = 12$) and more than 2 ng/ml (Group B, $n = 10$) PCT levels. The data were compared by Mann-Whitney U-test and Wilcoxon matched pairs test, p-value of < 0.05 was considered statistically significant. The data are expressed as median and 25th and 75th percentiles.
Results: None of the patients had exhibited any signs of infection before surgery. Initial PCT concentrations didn't exceed the normal values (< 0.5 ng/ml). The levels of 3h14 were significantly higher post-op compared to the baseline (6.1 (5–8.9) ng/ml vs. 5.6 (3.3–7.1) ng/ml, $p = 0.006$). Post-op 3h14 levels were 5.1 (4.9–7.6) ng/ml in Group A and 7.9 (5.6–9.6) ng/ml in Group B, $p = 0.038$. The rate of systemic inflammatory response syndrome (SIRS) on the 1st day post-op was also significantly higher in Group B (70% vs. 33% in Group A, p =0.04).
Conclusions: GC-MS method may be a suitable tool for detection of endotoxin chemical markers in blood plasma samples. Elevated levels of 3h14 are associated with the development of SIRS of bacterial origin, as confirmed by PCT-test.

P979

Are blood cultures necessary in the management of women with complicated pyelonephritis?

Y. Chen, O. Nitzan, W. Saliba, B. Chazan, R. Colodner, R. Raz *(Afula, Haifa, IL)*

Objectives: Data from previous studies suggest that blood cultures in women with uncomplicated acute pyelonephritis (APN) are of limited value. Our objective was to assess the role of blood cultures in the management of complicated APN in women, and to examine the demographic and clinical characteristics, and the outcome as related to the bacteraemic status of these patients.
Methods: Data from medical records of 158 women hospitalised with complicated APN over a 2-year period was analysed retrospectively. It included demographic, clinical and laboratory data, empiric antimicrobial therapy, urine and blood culture results, complications and clinical outcome.
Results: Out of 158 women with complicated APN, in 155 (98%) the urine culture grew pathogens, and 33 (20.9%) of them had bacteraemia. In the great majority of patients (98.7%), the blood cultures were sterile, or contained the same phenotypically profiled pathogen that was isolated from the urine. Only in 2 patients (1.3%), the blood cultures grew pathogens different from those found in the urine. The initial empiric antimicrobial therapy was not changed in any of the patients. No significant difference existed between the bacteraemia and non-bacteraemia patients in the demographic and clinical characteristics, the severity of disease or the outcome.
Conclusion: In the management of complicated APN, routine cultures of blood are often unnecessary.

P980

Procalcitonin marker for sepsis diagnosis

A. Emami Naeini, S. Montazerolghaem *(Isfahan, IR)*

Objective: Sepsis is a life-threatening syndrome and a common problem in all acute care hospitals. Routine laboratory tests lack both sensitivity and specificity in correctly identifying sepsis from other non-infectious systemic inflammatory response syndrome (SIRS). Most confirmatory microbiological test results are time consuming. The aim of this study is determining value of Procalcitonin (PCT) detection by a rapid, no instrument dependent test for differentiation patients with non-infectious SIRS and patients with sepsis, sepsis syndrome and septic shock.
Method: We conducted this prospective, cross-sectional, case control survey on patients with SIRS criteria with and without infection. The subjects were comprised 50 patients [32(64%) males and 18(36%) females] aged 18 to 94 years (mean: 56 ± 38). 25 in sepsis group with positive blood culture, [19(76%) male, 6(24%) female]. 25 SIRS group without infection [13(25%) male and 12(48%) female]. Patients admitted in St -Zahra hospital, Isfahan (centre of Iran) during February 2004 to February 2005. After taking 2 ml of venous blood sample from each patient at admission ward, we separated the serum, and stored the samples frozen at -20°C. Samples with severely haemoglobin values > 5 g/dl (haemolytic) were excluded. The PCT level was measured using a semi-quantitative immunochromatographic rapid test (B•R•A•H•S• PCT -Q B•R•A•H Diagnostica, Germany). All samples were examined after bringing to room temperature; a new individual test was used for each determination. Data were analysed by t student –test, and one-way analysis of variance using SSPS software version 11.
Results: 22 (88%) of those patients with sepsis had positive test (slightly elevated PCT = 0.5 ng/ml [3(12%)], moderately elevated PCT > 0.5 ng/ml [5(20%)], markedly elevated PCT ≥ 2 ng/ml [11(44%)], severely elevated PCT ≥ 10ng/ml [3(12%)] and 1 (4%) out of those with no sepsis SIRS had moderately elevated level of PCT.
Conclusion: In this survey PCT shows 88% sensitivity, 96%specificity and clearly discriminates sepsis from non-sepsis SIRS.PCT titre raises with progression toward sepsis syndrome and septic shock. PCT titre can aid in diagnosing, monitoring and outcome prediction in septic patients and can be considered as a routine and useful test in the initial workup of sepsis.

P981

Brain natriuretic peptide as diagnostic and prognostic marker in patients with bacterial infection and sepsis

A. Charalampopoulos, A. Stavrianou, C. Kotsovassilis, P. Tsiodra, C. Keramidas *(Athens, GR)*

Objectives: Brain natriuretic peptide (BNP) is a well-known index of left ventricular systolic dysfunction and a prognostic

marker in congestive cardiac failure and myocardial infarction. In critically ill patients such as those in sepsis and septic shock left ventricular dysfunction and subsequently high serum levels of BNP have been reported. Some studies suggest the potential prognostic value of BNP in patients with sepsis. The purpose of our study was to determine (a) if BNP serum levels increase in sepsis and (b) if BNP can predict outcome and be a valuable prognostic marker.

Methods: We examined retrospectively 70 patients with febrile bacterial infections admitted to our department over a period of one year. No one had a history of established cardiac failure. The diagnosis of the infection was based on clinical and laboratory data. Among the 70 patients 39 were in sepsis on admission. We measured BNP in blood samples taken the first two days of the hospitalization. The outcome was determined as survivors and non-survivors. Statistical tests applied were Student's t-test and Chi-Square test.

Results: We had 32 male and 38 female patients. In patients with sepsis the mean BNP value was 268.7 (SD: 271.3) and in patients without sepsis was 152.3 (SD: 198.3). There was a significant increase of BNP levels in patients with sepsis ($p < 0.05$). Among the 70 patients 55 were survivors (78.6 %) and 15 non-survivors (21.4 %). All the non-survivors were in severe sepsis. The percentage of death among the septic patients was 38.4 %. The mean BNP in survivors was 161.8 (SD: 197.87) and in non-survivors 420.2 (SD: 642.1) but that difference was not statistically significant ($p > 0.05$).

Conclusions: In our study BNP, measured soon after admission, (a) increases significantly in patients with sepsis and (b) does not seem to be a valuable prognostic marker in patients who suffer from febrile bacterial infections and sepsis. Further studies with more participants are needed to confirm that hypothesis.

P982

The value of the automatised blood culture systems in the diagnosis of CAPD peritonitis

Ö.K. Azap, F. Timurkaynak, S. Sezer, Ü. Cagir, G. Yapar, H. Arslan, N. Özdemir *(Ankara, TR)*

Objectives: Peritonitis is a common clinical problem that occurs in patients with end-stage renal disease treated by peritoneal dialysis (PD). Therapy should be guided by culture results and antimicrobial sensitivities. The aim of this study is to evaluate the value of the blood culture systems in the diagnosis of CAPD (continuous ambulatory peritoneal dialysis) peritonitis.

Methods: Twenty-six samples of peritoneal fluid obtained from patients with the suspicion of CAPD peritonitis were included. Leukocyte count, Gram and Wright stains of the samples were performed. All samples were both inoculated onto solid agar media and into the blood culture broth (BACTEC 9050®). Agar plates were incubated for 48 hours and the blood culture bottles were incubated for 5 days unless a positive signal was obtained.

Results: Leukocyte count was $< 100/mm^3$ in 9 of the 26 samples and the cultures of these samples grew no bacteria. Of the 17 samples, which have leukocyte counts $> 100/mm^3$ 14 samples grew in solid agar and/or blood culture media. Etiologic agent was detected in both blood culture broth and on the agar plates in 4 samples; on the other hand in 8 samples bacteria grew only in blood culture broth and in two samples only on solid agar media in 2 samples. The two growths on only solid agar media were interpreted as contamination. As a result significant growth was detected in 12 (70.5%) of 17 samples. The most striking finding is that 8 (66.6%) of these 12 results were obtained only from blood culture bottles. The identified pathogens are methicillin-sensitive coagulase-negative *staphylococci* (in 5 of the samples), alpha-haemolytic *streptococci* (in 2 of the samples), *Corynebacterium* spp. (in 2 of the samples), *Escherichia coli* (in 2 of the samples), *Enterococcus faecalis* (in 1 of the samples).

Conclusion: Infectious complications account for approximately two thirds of all PD catheter losses. Treatment should be based on antimicrobial susceptibility results. This mandates the proper management of the samples obtained from CAPD patients. The use of blood culture bottles inoculated with peritoneal fluid at the bedside, rather than submitting the specimen to the laboratory for later processing is advocated.

P983

No beneficial impact of shortened microbiological procedures for either hospitalized patients overall or for patients with bacteraemia

M.J. Bruins, H.C.A. Oord, P. Bloembergen, M.J.H.M. Wolfhagen, A.F. Casparie, J.E. Degener, G.J.H.M. Ruijs *(Zwolle, Rotterdam, Groningen, NL)*

Objectives: Shortening the turnaround time of microbiological procedures by using an automated system for bacterial identification and susceptibility testing is associated with an improved clinical outcome and a reduction of hospital cost, according to two American studies. We investigated whether the same beneficial effects could be reported for patients in a hospital in the Netherlands by conducting a single blind, randomized controlled trial.

Methods: Patients hospitalized in the Isala klinieken in Zwolle, the Netherlands, with a bacterial infection confirmed by culture were randomly assigned to a control or to an intervention (rapid) group. As is customary practice, the clinical microbiologists orally reported clinically relevant information to the clinician for all patients. For all patients complete culture results were reported on paper. For identification and susceptibility testing overnight methods were used in the control group. In the rapid group the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) was used. In each of the three consecutive study periods accelerating factors were added in a step-up manner to the laboratory workflow of the rapid group, such as increasing oral reporting, extending the working day and adding an extra hard-copy report delivery. The workflow for the control group remained identical throughout the study. We analysed whether the turnaround time to reporting was shortened by using the Vitek 2 system and additional factors, and assessed the effects of a shortened turnaround time in terms of mortality, morbidity and cost for (i) the overall patient group and (ii) a subgroup of patients with bacteraemia

Results: For the overall patient group ($n = 1870$), the time interval to oral reporting of final susceptibility results was significantly shorter for the rapid groups in all three-study periods, the time to reporting on paper was significantly shorter in the third period. For the subgroup of patients with bacteraemia (n = 183) a similar shortening of turnaround times was found. For neither the overall patient group nor the subgroup a significant difference in any of the clinical impact variables or a cost reduction was found.

Conclusion: Although the turnaround time to reporting of microbiology results was shortened significantly for both the overall patient group and the subgroup of bacteraemic patients, in our hospital setting no clinical impact or cost reduction could be reported as a result.

P984

Routine use of the *Bac*T/ALERT FAN anaerobic bottle: indicated or not?

B. Van Meensel, J. Verhaegen, J. Frans, H. Castryck, N. Blanckaert *(Leuven, BE)*

Objectives: Traditionally, routine blood cultures for adult patients consist of paired aerobic and anaerobic blood culture bottles. However, expert opinions differ on the routine use of the anaerobic blood culture bottle, debating substitution of the anaerobic bottle by an additional aerobic bottle. In this way, the optimal volume of 20 ml of blood per culture for detecting bacteraemia would be collected. The aim of our study was to evaluate the benefit of routinely using the combination of the *Bac*T/ALERT FAN aerobic and anaerobic bottle in a tertiary care medical centre.
Methods: We performed a retrospective study of 11 740 blood culture bottles (5855 paired and 30 unpaired) obtained from 3611 adult patients admitted to the emergency room between January 2005 and August 2005. Statistical analysis using the sign-test was limited to those isolates classified as clinically significant and was only performed when at least 10 isolates per category were recovered.
Results: A total of 325 significant microbial strains were isolated from 312 patients. Analysis of these isolates revealed no significant difference between the yield of the FAN aerobic and FAN anaerobic bottles. However, significantly more *Pseudomonas aeruginosa* ($p < 0.0001$) and *Streptococcus pneumoniae* ($p = 0.0156$) were recovered from the aerobic bottle, while significantly more strictly anaerobic bacteria ($p < 0.0001$) and *Enterobacteriaceae* other than *Escherichia coli* ($p = 0.0156$) were recovered from the anaerobic bottle. Twenty-two patients (7.1 %) had a positive blood culture with strictly anaerobic bacteria, which were only recovered from the anaerobic bottles. These were mainly elderly patients, 15 with an abdominal pathology, 3 with neutropenic fever, and 3 with a decubitus ulcer and 1 with a tooth abscess. In two of these 22 patients, the result of the blood culture led to a change in diagnosis and an adjustment of the antibiotic therapy.
Conclusion: Our data suggest that the routine use of the *Bac*T/ALERT FAN anaerobic bottle is still recommended in our institution because we have a relative high proportion of strictly anaerobic bacteraemia and because some facultative organisms apparently grew preferentially in the anaerobic bottle. Furthermore, although infrequently, the knowledge of an anaerobic bacteraemia did influence the management of patients.

P985

Developing clinical rules and a urinary dipstick rule to predict urinary tract infection in primary care settings

P. Little, S. Turner, K. Rumsby, G. Warner, M. Moore, H. Smith, C. Hawkes, M. Mullee *(Southampton, Hastings, UK)*

Background: Suspected UTI is one of the commonest acute infections. Systematic reviews have documented no adequately powered studies in primary care to assess independent predictors of rigorous laboratory diagnosis, nor robustly estimated the performance of urinary dipsticks in a typical primary care population. Our aim was to estimate independent clinical and dipstick predictors of infection and develop clinical decision rules.
Methods: 427 women presenting with suspected UTI in primary care had laboratory diagnosis assessed using the European Urinalysis guidelines standard. Independent clinical and dipstick predictors of diagnosis were estimated.
Results: 62.5% of women had confirmed UTI. Only nitrite, leukocyte esterase (+ or greater), and blood (haemolyzed trace or greater) independently predicted diagnosis (multivariate odds ratios respectively 6.36,4.52,2,23). A dipstick rule – based on having nitrite, or both leukocytes and blood was moderately sensitive (77%) and specific (70%) (positive predictive value (PPV) 81%, negative predictive value (NPV) 65%). Predictive values were improved by varying the cut point: the NPV was 73% for all three-dipstick results being negative; the PPV 92% for having nitrate and either blood or leukocyte esterase. A 'clinical rule' based on having 2 of urine cloudiness, offensive smell, reported moderately severe dysuria, moderately severe nocturia - was less sensitive (65%) (Specificity 69%, PPV 77%, NPV 54%). The NPV was 71% for none of the four clinical features, and the PPV 84% for 3 or more features.
Conclusion: Simple decision rules could improve targeting of investigation or treatment. Strategies to use such rules will need to take account of their limited sensitivity, which is lower than expected from previous research.

P986

Quantitative sputum culture versus direct sputum culture

O. Akan, E. Ozyilmaz, K. Ahmed, S. Uysal, M. Gulha *(Ankara, TR)*

Objectives: Gram stain is preferred for the diagnosis of lower respiratory tract infections (LTRI) at microbiology practice, but sputum culture is still needed to isolate the pathogens for antibiotic susceptibility tests. This study is performed to evaluate the quantitative sputum culture (QSC) compared to direct sputum culture (DC) for isolation of the common bacteria causing LTRI.
Methods: 100 sputum obtained from adults with or without chronic obstructive lung disease having clinically and/or radiologically diagnosed LRTI were included in the study. All of the samples contained ≥ 25 polymorphonuclear leucocytes (PNL), and < 10 epithelial cells per low-power field (LPF) under light microscopy and all of them revealed at least one microorganism as a causative agent by Gram stain. After direct inoculation of culture plates, the sputum samples were mixed with Sputasol (Oxoid) at a ratio of 1:1 and after serial dilutions, quantitative cultures were performed. Blood and chocolate agar plates were used for culture and standard microbiological methods were used for bacterial identification. 24 and 48 hours later both direct plates and quantitative cultures were observed. Cut off point ≥ 1000 000 cfu/ml was determined to be positive for the QSC.
Results: 16 % of sputum samples were negative by both culture techniques. Ten cultures displayed mixed growths with two organisms and 94 bacteria from 84 positive sputum cultures were observed. *Haemophilus influenzae* (48%) was the most common isolate followed by *Streptococcus pneumoniae* (32%) and *Branhamella catarrhalis* (14%). QSC detected 97.9% and DC detected 60.6% of the pathogens. 39.4% of bacteria (16 *H.influenzae*, 18 *S.pneumoniae*, 3 *B.catarrhalis*) were detected by QSC only and this rate was 2.1% (1 *Branhamella catharralis*, 1 *S.pneumoniae*) for DC. QSC detected more agents than DC ($p < 0.0001$).
Conclusion: To obtain pathogenic bacteria from sputum samples, which are available for culture, QSC can be used instead of DC. For routine use of QSC at clinical microbiology laboratories, cost effective analysis should be performed according to the individual needs of the health care facilities.

P987

Quantitative urine microscopy as a quick, reliable examination for bacteriuria

S. Mhalla, A. Ferjani, N. Hannechi, J. Boukadida *(Sousse, TN)*

Objectives: The diagnosis and treatment of urinary infection are often delayed, causing renal damage, largely because of the unavailability of quick, accurate, diagnostic examination. The aim of this study is to compare the accuracy in diagnosing significant bacteriuria between quantitative urine microscopy and the Gram-stain method.
Methods: Urine samples were obtained from fresh urine specimens, which were sent to our laboratory during the month of October 2005.These urine samples, were examined for significant bacteriuria using the standard culture, than examined using the Gram-stain method and quantitative urine microscopy by Mallassez cell. When particles could not be distinguished definitely as bacilli by quantitative microscopy, the urine was examined using oil immersion microscopy at × 1000 magnification.
Results: Significant bacteriuria was detected by bacterial culture in 70 of 325 urine samples. Using quantitative microscopy, rods were found in 52, *cocci* in a cluster in 2, *cocci* in a chain in one, and indefinite particles in 19 samples. In the 19 indefinite samples, oil-immersion microscopy was able to distinguish rods in 9, *cocci* in a cluster in one, and negative in 9, which were confirmed by culture as rods, *staphylococci* and negative, respectively. The quantitative microscopy method was more reliable (93% sensitivity, 98% specificity) for diagnosing of significant bacteriuria when compared with the Gram-stain method (70% sensitivity, 93% specificity).
Conclusion: Quantitative urine microscopy, confirmed by oil-immersion, is a quick, reliable method for diagnosis of significant bacteriuria, and is considered to be useful for early diagnosis of urinary infection.

P988

Detection of extended-spectrum beta-lactamases among *Enterobacteriaceae* using automated microbiology systems and manual detection procedures

H. Seifert, H.K. Geiss, D. Mack, E. Stürenburg, I. Wiegand *(Cologne, Heidelberg, Hamburg, Bonn, DE; Swansea, UK)*

Objectives: The reliability of automated antibiotic susceptibility testing systems for the detection of extended-spectrum beta-lactamases (ESBLs) among *Enterobacteriaceae* is crucial for optimal patient therapy and outbreak management.
Methods: A total of 150 strains, including *Escherichia coli* (62), *Klebsiella* spp. (45), *Enterobacter* spp. (24), *Citrobacter* spp. (6), *Morganella morganii* (4), *Proteus* spp. (7), and *Serratia marcescens* (1) were tested. Three automated microbiology systems were compared: The Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD), the VITEK® 2 system (bioMérieux, Marcy l'Etoile, France), and the Microscan WalkAway®-96 SI (Dade Behring, Inc.). Conventional phenotypic confirmatory test such as the NCCLS double disk synergy test (DDS), the disk approximation method (DAM), and the Etest ESBL (AB biodisk, Solna, Sweden) were also evaluated. Combined uses of isoelectric focusing, PCR procedures for the detection of TEM and SHV genes, and/or DNA sequencing were used as the reference method.
Results: Of the total 150 isolates, 95 were ESBL producers as determined by the reference methods, 2 isolates were excluded due to indeterminate results, and the remaining 53 isolates were identified as non-ESBL producers. The sensitivity, specificity, negative (NPV) and positive (PPV) predictive values were determined:

	Phoenix	Vitek 2	WalkAway	DDS	DAM	Etest ESBL
Sensitivity	91.6	80.0	84.2	86.3	88.4	91.6
Specificity	79.2	79.2	73.6	98.2	83.0	90.6
NPV	84.0	68.9	68.9	80.0	80.0	85.7
PPV	88.8	87.4	87.4	98.8	90.3	94.6

Conclusions: These results indicate that the Phoenix System offers the highest sensitivity and acceptable specificity among the automated susceptibility testing systems for the detection of ESBL production in clinically important *Enterobacteriaceae*. The most reliable overall performance was achieved with the ESBL ETest.

P989

Assessment of the delay in reporting blood culture results – development of a method to identify defects in quality

U. Saarela, P. Kärpänoja, H. Sarkkinen *(Lahti, FI)*

Objectives: It is a well-known fact that early reporting of positive blood cultures results in a more rational treatment of patients with sepsis. One might expect that early reporting of a negative blood culture as well as impact on the clinical assessment and treatment of patients with symptoms of infection. We have previously analysed that 93 % of all positive blood cultures are flagged as positive in our automated blood culture system within two days of incubation. Based on that, we have defined the maximal delay time of two days for the first report of a blood culture, whether positive or negative. To verify that our laboratory practice complies with this guideline, we audited our blood culture reports.
Methods: 10 001 blood culture reports were included in the analysis. The blood culture bottles were incubated in the *Bac*T/Alert® (Bio-Merieux) automated blood culture system. 813 (8.1 %) of these were positive. According to our documented procedure positive results are reported by phone and the laboratory information system (Effica Microbiology System®, TietoEnator) immediately after gram-staining and preliminary identification directly from the blood culture bottle. Negative results are reported for the first time after two days of incubation and finally after five days. The implementation of the reporting system (expected reporting delay) was analysed using a developed report generator, now included in the Effica Microbiology System® software. To exclude the various variations due to the workflow in the laboratory, the acceptable delay was set to three days instead of two.
Results: The mean delay time in reporting the first result (whether positive or negative) was 1.45 days with a standard deviation of 0.737. The median was 1.00 days (minimum 0 days, maximum 25 days). 9913 (99.1 %) of all results were reported within the three days. We found 88 (0.9 %) cases where reporting delays exceeded the pre-defined time. Seven of these reports were not considered errors on different grounds. For the 81 cases no acceptable reason for the delay was found. They were regarded as human errors.
Conclusions: The reporting delay is an important quality indicator in a microbiological laboratory. The number of results reported falsely later than three days after the beginning of the incubation, was very small (n = 81, 0.81 %). With the developed reporting generator described we are able to detect the ''outliers'' thus improving the quality of our reporting.

P990

Validation of commercial kits for detection of antibodies to *Legionella*

P. Elverdal, S.A. Uldum *(Copenhagen, DK)*

Objectives: Validation of four commercial kits for detection of antibodies to *Legionella* (L.) *pneumophila* by comparison with an in-house *Legionella* IFA assay. Three-kits were indirect Enzyme-Linked Immunosorbent Assays (ELISA) and one kit was an indirect immunofluorescent antibody assay (IFA). For this study, the kits were tested with sera, which had been tested positive for other bacteria than *Legionella*, and with in-house IFA assay positive sera from patients with *L. pneumophila* infection.
Methods: Seventy-five sera, which had been tested positive for other bacteria (*Campylobacter*, *Helicobacter*, *Riskettsia*, *Mycoplasma pneumonia*, *Chlamydia* group, *Pseudomonas aeruginosa* and *Coxiella*) than *Legionella*. One hundred in-house IFA assay positive sera (titre ≥ 256) from patients with *L. pneumophila* infection confirmed by one of the following methods: PCR, culture or *Legionella* urinary antigen test. In the in-house IFA assay heat inactivated *L. pneumophila* serogroup 16, *L. micdadei* and *L. bozemanii* were used as antigens. The kits, which were validated, were:
(i) Focus Diagnostics IFA kit for *L. pneumophila* serogroup 1–6 and 8,
(ii) SERION ELISA classic for *L. pneumophila* 1–7 IgM kit,
(iii) SERION ELISA classic for *L. pneumophila* 1–7 IgG kit, and
(iv) Zeus Scientific, Inc. *L. pneumophila* serogroup 1–6 ELISA kit
Results: In Table 1, it can be seen that the sensitivity was highest for the Focus Diagnostics kit (77%), but this kit also had the next highest false positive rate properly caused by cross-reaction (16%). To compare the SERION ELISA classic kits with the other kits, the IgM and IgG results were combined because the other kits all detect IgG/IgM/IgA. The SERION ELISA classic kits had a sensitivity of 73.9% and a false positive rate of 5.6%, when the two kits (IgM + IgG) results were combined. Table 1: Numerical percent of sensitivity and cross-reaction in kits and LAT.

		Sensitivity (%)	False positive rate (%)
Legionella Antibody test (LAT, in-house IFA)	IgG/IgM/IgA	100	5.3
Focus Diagnostics kit (IFA)	IgG/IgM/IgA	77.0	16.0
SERION ELISA classic kit	IgM	51.2	5.3
	IgG	53.8	2.8
	IgM+IgG	73.9	5.6
Zeus Scientific, Inc. ELISA kit	IgG/IgM/IgA	72.5	29.0

Conclusion: The Focus Diagnostics, SERION ELISA classic and Zeus Scientific, Inc. ELISA kits are all relatively sensitive. But both Focus Diagnostics and Zeus Scientific, Inc. ELISA kits have high false positive rates. The best kit, compared to the in-house assay, is the SERION ELISA classic kit when the IgM and IgG results are combined, because it has the best combination of a relative high sensitivity and a low false positive rate.

P991

Intrathecal synthesis of anti-borrelia antibodies in multiple sclerosis

J. Bednarova, P. Stourac *(Brno, CZ)*

Objectives: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of autoimmune origin. It is the most common cause of neurologic disability in young adults. The intrathecal synthesis of specific IgG antibodies against measles, rubella and varicella zoster viruses, called MRZ reaction, is reported in 84–94% MS patients. As a part of oligoclonal, polyspecific immune response in MS, intrathecal synthesis of specific IgG antibodies against herpes simplex virus and toxoplasma is also reported. The aim of our study was to investigate the frequency of intrathecal synthesis of specific IgG antibodies against *Borrelia burgdorferi* in MS.
Methods: We investigated a cohort of 101 patients: 56 patients were diagnosed as multiple sclerosis, 30 patients were diagnosed as neuroborreliosis (NB), 15 patients had other neurologic diseases (OND). Serum and CSF samples were analysed at each patient. The diagnostic kit of Test-Line Company, Clinical Diagnostics, Czech Republic (EIA *Borrelia garinii* IgG) was used for the detection of specific antibodies. Absolute values of absorbances were converted to arbitrary units (AU) and the intrathecal synthesis was evaluated as specific antibody index (AI) according to Reibers method. Values of AI > 1.4 indicated positive intrathecal synthesis. Statistical significance was confirmed by Spearman rank coefficient and Wilcoxon test.
Results: Intrathecal synthesis of specific IgG antibodies against *B.burgdorferi* was detected in 25% MS patients with AI in the range 1.8–9.3. The CSF arbitrary units of antibodies against *B.burgdorferi* were in the range 0.5–65.0 with AU median 1.8 and AU mean 7.2. Intrathecal synthesis of specific IgG antibodies against *B. burgdorferi* was detected in 90% NB patients with AI in the range 1.5–33.0. The CSF arbitrary units of antibodies against *B.burgdorferi* were in the range 4.0–100.0 with AU median 100.0 and AU mean 75.2. One-OND patient had positive intrathecal synthesis against *B.burgdorferi* with AI = 2.5. Low anti-borrelia AU in MS patients reflect polyspecific immune response in MS compared to high anti-borrelia AU in NB patients that indicate immune response against *B.burgdorferi* as the causative agent in NB.
Conclusion: We detected positive intrathecal synthesis of specific IgG antibodies against *B.burgdorferi* in 25% MS patients. Specific intrathecal synthesis against infectious agents in MS is a part of polyspecific immune response in chronic autoimmune disease of the nervous system.

P992

Antimicrobial susceptibility as a complementary diagnostic tool for the identification of rarely encountered non-fermenting Gram-negative bacteria

Y. Glupczynski, G. Detry, C. Berhin, M. Janssens, G. Wauters *(Yvoir, Brussels, BE)*

Objectives: Gram-negative non-fermenters (GNNF) other than *Pseudomonas aeruginosa* and *Acinetobacter* spp. are increasingly implicated in various different types of nosocomial infections. Commercially available identification methods have limited performances and conventional methods despite improved accuracy are difficult to implement in routine diagnostic laboratories. Because of the occurrence of the wide variations in the natural antimicrobial resistance patterns among the different genus and/or species of rarely encountered GNNFs, we aimed to assess the antibiogram as an auxillary aid in their identification.
Methods: 218 clinical strains and 34 reference strains belonging to 31 different genera and species of rarely encountered GNNFs identified to the species level by conventional methods and/or by sequencing or 16S rRNA gene were tested by disk diffusion against 30 to 32 agents representing most classes of antibiotics. Susceptibility categorization was made according to CLSI interpretative criteria for non-fermenters except for colistin

which was interpreted as resistant on the basis of the absence of any zone size.

Results: *S. maltophilia*, *C. meningosepticum* and *O. anthropi* displayed the most multi-resistant profile. Besides colistin and fosfomycin, the susceptibility or resistance pattern to specific beta-lactam agents (see table) was found as a useful complementary tool for identification: Susceptibility to aztreonam and to temocillin was only found in *Burkholderia, Deftia* and *Comamonas* species. Resistance to carbapenems sometimes dissociated between imipenem and meropenem was encountered in *Stenotrophomonas*, *Chryseobacterium*, *Burkholderia* and *Ralstonia*. Synergy between beta-lactamases inhibitors and beta-lactams was only seen with *Stenotrophomonas*, *Chryseobacterium*/*Myroides* spp. and *Rhizobium* spp. Images of cephalosporinases induction by cephamycins, carbapenems or beta-lactam/beta-lactamases inhibitors was typically seen in *Ochrobactrum anthropi*.

Species	AMC	PTZ	TEM	AZT	CTX	MER	IMP	COL	FOS
S. maltophilia	R	R	R	R	R	R	R		R
B. cepacia	R						R	R	R
R. pickettii	R		R	R		R		R	R
A .faecalis			R	R					R
A. xylosoxidans			R	R	R				R
D. acidovorans								R	R
Comamonas spp.				R					R
O. anthropi	R	R	R	R	R				R
C. meningosepticum	R	R	R	R		R		R	R

Conclusion: Overall the natural antibiotic resistance patterns of several GNNF genus/species group appears may appear as a valuable useful complementary for identification of these organisms.

P993

An improved protocol for the serological diagnosis of atypical pneumonia

C. Jenkins, I. Balakrishnan *(London, UK)*

Objectives: The Royal Free Hospital Microbiology Department receives approximately 500 requests for atypical pneumonia serology tests each year. Prior to April 2005, sera from all atypical pneumonia serology requests were analysed for antibodies to *Mycoplasma pneumoniae, Chlamydia pneumoniae* and *Coxiella burnetti*. (*Legionella pneumophila* is detected using a urinary antigen test and not included in this study.) However, tests on acute-stage sera can neither exclude a specific aetiology by a negative result nor firmly establish it by detecting antibody in an early sample. Tests on paired sera are more useful but the inherent delay reduces the value of a result for case management.

Methods: From April 2005, clinicians were requested to provide details on the date of onset of patients' symptoms. Samples taken less than seven days from the onset of symptoms or those with no date of onset were not tested but saved at -20°C for seven days and a report issued stating 'Serum saved. Please phone Microbiology if specimen was taken more than 7 days into illness or send follow-up specimen in 7–10 days for parallel testing if indicated. The requests for atypical pneumonia serology from 01.04.04 to 30.09.04 were compared to those sent between 01.04.05 to 30.09.05 to determine whether the new protocol had reduced the number of tests performed and improved the service provided.

Results: During the review period, 159 requests for atypical pneumonia serology were sent in 2004 compared to 309 in 2005. Sixty-six of the requests received in 2005 were not tested immediately as they did not fulfil the testing criteria. Of these, 27 were tested after the clinician provided the required information. No feedback was received from the requesting clinician in 39 of these samples and they were not tested. The Infectious Diseases ward responded with further information most often whereas Accident and Emergency were least likely to respond.

Table 1. Comparison of the number of atypical serology tests performed in 2004 with those carried out in 2005 during the same period.

Assay	2004	2005
Total number of requests for atypical serology	159	309
Mycoplasma pneumoniae	97 (61%)	163 (53%)
Chlamydia pneumoniae	21 (13%)	41 (13%)
Coxiella burnetti	35 (22%)	39 (13%)
Samples saved and date of onset of symptoms requested	-	66 (21%)
Date of onset provided	-	27 (41%)
No information received	-	39 (59%)

Conclusion: From April to September 2005, tests were not performed on 39 samples due to the change in protocol, resulting in a reduction in both expenditure and workload. There was no significant reduction in the number of requests sent without the date of onset of patients' symptoms, despite our proactive approach. However, there was an increase in the number of clinicians responding to our request for more information. In addition, the new protocol has improved the quality of the service by rationalizing the diagnosis of atypical pneumonia.

P994

Potential antigenic determinants of *Chlamydia trachomatis* major outer membrane protein modeled by overlapped recombinant proteins

N. Savel'eva, J. Zagriadskaya, S. Klimashevskaya, V. Puzyrev, A. Obriadina, A. Burkov, T. Ulanova *(Nizhnyi Novgorod, RU)*

Background: Major outer membrane protein (MOMP) is a primary target of specific anti-*Chlamydia trachomatis* antibodies. This protein performs important structural and immunity-related roles and contains 4 variable segments (VS I, II, III, IV). Previously it has been shown that immunoreactive antigenic epitopes are located within VS I, II and IV.

Objective: The purpose of this study was determination of potential antigenic epitopes encoded by open reading frame for major outer membrane protein of *Chlamydia trachomatis* and modelling them by using overlapped recombinant proteins about 100 amino acids (aa).

Methods: Several antigenic determinants of *Chlamydia trachomatis* MOMP have been predicted by bioinformatics analysis. Six pairs of primers were designed to produce six overlapping DNA fragments from genomic DNA *Chlamydia trachomatis* by using PCR reaction. Recombinant genes encoding selected amino acid sequences about 100 aa with overlapping 30 aa have been synthesized. Proteins were expressed in *Escherichia coli* as hybrid proteins with Glutathione S-transferase and 6-Histidine tag. To study antigenic properties of new proteins 64 well defined positive (n = 39) and negative (n = 25) serum samples were tested. All serum samples were previously characterised by three commercially available assays for the detection of IgG anti-*Chlamydia trachomatis*.

Results: Six clusters of potential antigenic determinants have been predicted within major outer membrane protein of *Chlamydia trachomatis*. Recombinant genes encoding predicted amino acid sequences of MOMP at positions 1–116 aa, 66–165 aa, 128–216 aa, 191–286 aa, 252–354 aa and 317–398 aa were synthesised. The pure samples of 6 proteins were obtained by affinity chromatography. All proteins were immunoreactive and demonstrated different specific activity with anti-*Chlamydia trachomatis* antibodies in serum samples. The 191–286 aa protein showed the highest level of immunoreactivity. This antigenic epitope(s) comprise VS III.
Conclusion: The predicted antigenic epitope(s) located within 191–286 aa demonstrated a significant diagnostic potential as candidates for the development of diagnostic assays for the detection of anti-*Chlamydia trachomatis* IgG activity in serum specimens.

P995

Evaluation of the Giardia-strip: an *in vitro* immunochromatographic test for the detection of *Giardia lamblia* cysts in faecal specimens

D. Van Kerkhoven, M. Lontie, J. Verhaegen, K. Lagrou *(Leuven, BE)*

Objectives: Giardiasis is a diarrhoeal illness caused by *Giardia lamblia*, a one-celled parasite that lives in the intestine of humans and animals. The disease is diagnosed by microscopical identification of cysts or trophozoites in faeces, using direct mounts as well as concentration procedures. Since these analyses are labour-intensive and require a skilled microscopist, antigen detection tests (direct fluorescent antibody (DFA), enzyme immunoassay (EIA) and rapid, dipstick-like tests) have been developed as alternatives. We have evaluated the performance of a commercially available one-step immunochromatographic membrane test using specific monoclonal antibodies against the cyst membrane antigens of *G. lamblia*, the Giardia-strip (CorisBioconcept).
Methods: The test performance was evaluated with known positive (n = 18) and negative (n = 55) stool specimens for *G. lamblia*, tested by the standard ova and parasite (O&P) examination as the golden standard. Faeces with other parasites (8 *Endolimax nana*, 8 *Entamoeba coli*, 4 *Entamoeba histolytica* and 1 *Ancylostoma*) and *Staphylococcus aureus* (3) were included to evaluate the specificity of the test. These fresh and unpreserved samples, obtained from the laboratory of UZ Gasthuisberg and Medisch Centrum voor Huisartsen Leuven, were frozen and maintained at -20°C prior to testing. The Giardia-strip was used according to the manufacturer's instructions.
Results: By the Giardia-strip, 17 of the 18 known positive specimens were positive and 54 of the 55 Giardia-negative samples were negative (94.4% sensitivity, 98.2% specificity, 94.4% positive predictive value and 98.2% negative predictive value), compared to (O&P) which is considered as the reference method. The Giardia false-positive discrepant sample came from a patient who was on holiday in Tunisia. Microscopy on a control sample of the same patient remained negative. The missed positive specimen by the Giardia-strip contained many cysts. No cross-reactions with other parasites or *S. aureus* were observed in this study.
Conclusion: The Giardia-strip has an excellent sensitivity and specificity for the detection of *G. lamblia* in stool. The test is easy to perform (no concentration prior to testing), suitable for single sample analysis and has a short turn-around-time (15 minutes). This diagnostic kit may be very beneficial in the absence of trained microscopists. However, it cannot substitute the routine O&Ps as only *G. lamblia* is detected.

P996

Use of the T tube test in the setting of a paediatric tuberculosis clinic

J. Paton, C. Lucas, C. Williams *(Glasgow, UK)*

Objective: To assess the feasibility of use and performance of the T Spot TB test within a paediatric TB clinic by monitoring the level of agreement between tuberculin skin testing (TST) and T Spot TB test.
Methods: T-SPOT (Oxford Immunotec) directly detects individual activated effector T-cells, via their secretion of the cytokine ã-Interferon using an ELISPOT (Enzyme Linked Immunosorbent Spot) assay. The assay is based upon the principle that T cells of individuals sensitized with tuberculosis antigens produce ã-interferon when they re-encounter mycobacterial antigens. Antigens specific to *M. Tuberculosis* such as early secretory antigenic target 6 (ESAT6); culture filtrate protein 10 (CFP10) are used to stimulate cells removed from the patients blood. These are more specific to *M. tuberculosis* than PPD, as they are not shared with any BCG sub-strains or most Non *Tuberculous Mycobacterium* (NTM) species with the exception of *M. kansasii*, *M. marinum*, and *M. szulgai*. We have performed 147 Elispot tests and have results comparing ELISPOT and TST in 65 cases.
Results: There was a technical failure of the test in 11 cases the commonest cause (8 tests) being insufficient cells to proceed with the assay. There was generally good agreement between negative ELISPOT and TST in patients with no history of BCG vaccination with agreement in 95% of cases, however in patients with positive TST only 54% were ELISPOT positive. In 13 patients with a history of BCG vaccination and positive TST only 1 was positive by ELISPOT.
Conclusions: The use of the T SPOT test is practical in a paediatric TB clinic. The assay has a high degree of concordance in negative tests in the absence of BCG vaccination and is also useful when previous BCG vaccination has been given however ongoing evaluation of this methodology in the paediatric outpatient setting is needed.

P997

Do hyaluran levels determine the outcome of bacterial infections?

H. Lydatakis, I. Hager, I. Binikos, P. Afentra, H. Chrisanthidou, T. Peppas, S. Pappas, I. Diamantis *(Piraeus, Athens, GR)*

Objectives: Bacterial infections remain a major cause of morbidity and mortality, despite the continuous development of antibiotics. The prognosis of these infections is variable and depends on host and bacterial specific factors. The role of CRP and WBC count as prognostic factors is controvercial. In this study, we measured the Hyaluran (HA) levels in an effort to find new prognostic factors which could define the clinical outcome of bacterial infections.
Patients/Methods: From June to October 2005, 54 immunocompetent adults, with confirmed bacterial infection, including cholecystitis, pneumonia and pyelonephritis, were admitted to our hospital. These patients were monitored for at least 9 days and the levels of HA, CRP and WBC count, as well as the temperature were measured on the 2nd and 8th day of hospitalization. The levels of HA were measured using an ELISA. The normal limits were 5–70 ng/ml. On the 2nd day, 32

patients had HA levels higher than 70 ng/ml (210.25 ± 170.12-Group 1), while the rest 22 had HA levels within normal range (48.73 ± 37.98-Group 2). Both groups had similar mean values of age, admission temperature, and admission serum CRP and WBC count.
Results: By the 8th day of hospitalization 28 patients of group 1 had complete remission of their infection, 2 developed septic syndrome and 2 died (6.2% mortality), while only 4 patients of group 2 improved, 11 developed septic syndrome and 7 died (31.8% mortality). The WBC levels of group 1 were significantly lower than that of the second group (15 300 ± 2137 vs.7850 ± 2116 $p = 0.001$). Likewise there was a significant reduction of serum CRP in the first group compared to the second one (33.30 ± 25.22 vs.180.83 ± 56.64 p=0.01). The temperature of patients of group 1 returned to normal limits (36.72 ± 0.23) while in group 2 remained high (38.21 ± 1.12) ($p = 0.003$). The mean HA levels remained high in patients of group 1 (135.89 ± ±33.12) while in patients of group 2 remained within normal limits (52.47 ± 28.12) ($p = 0.001$).
Conclusions: These data indicate that HA could be used as prognostic factor in determining the outcome of bacterial infections. More studies are needed to confirm these findings and to establish the mechanism by which the HA might influence the evolution of the bacterial infections. Finding the exact mechanism could help in designing new therapeutic strategies.

P998

Evaluation of the new chromogenic medium: CHROMagar *Salmonella Plus* for the detection of all *Salmonella* species including *Salmonella typhi* and *Salmonella lactose plus*

A. Rambach, Q. Tran, A.C. Le Dantec *(Paris, FR)*

Infections due to *Salmonella* continue to be a major problem throughout the world. Culture media have several advantages in comparison to PCR and immunological methods: culture media allow antibiotic susceptibility studies, epidemiological studies of the detected strains and, in addition, only living bacteria are detected. Recently several new chromogenic media improve the detection of *Salmonella*. We studied with pure strains the new chromogenic media CHROMagar *Salmonella Plus* (CHROMagar, France), which after 24 hours of incubation at 37°C detects *Salmonella* species including *Salmonella Typhi* and *Salmonella lactose plus*. Our first results, in comparison to Hektoen Enteric Agar (BD) and OSCM (Oxoid), show that only CHROMagar *Salmonella Plus* allows detection of *Salmonella* spp, *Salmonella Typhi* and *Salmonella lactose plus* while HEA and OSCM fail to detect many lactose positive *Salmonella*. Preliminary results show that CHROMagar *Salmonella Plus* is a convenient, rapid, sensitive and specific medium for detection of the three types of *Salmonella* species. The next step of the study will be the evaluation of CHROMagar *Salmonella Plus* for routine detection.

Bloodstream infections

P999

Characteristics of healthcare-associated bloodstream infections in adults in Korea

Y.K. Kim, S.Y. Shin, M.S. Kim, Y.S. Park, Y-A. Kim, H.J. Yoon, J.Y. Choi, Y.G. Song, K.H. Roh, D. Yong, K. Lee, J.M. Kim *(Seoul, KR)*

Background: The definition of community-acquired infection (CAI) needs to be reformulated with increase in outpatient management, nursing care facility, etc. The characteristics of healthcare-associated infection (HCAI) are similar to that of nosocomial infection (NCI) in several studies. We investigated the characteristics of HCAI caused by *S. aureus* and *E. coli*.
Methods: Four hundreds twenty two adult patients, admitted from Jan. 2004 to June 2005 at Severance Hospital, Seoul, Korea with bloodstream infection (BSI) caused by *S. aureus* or *E.coli* were studied. To characterize healthcare-associated bloodstream infections caused by *S. aureus* or *E.coli*, we investigated comorbid medical condition, predisposing factor, site of infection, resistance patterns, in-hospital mortality by epidemiological category such as NCI, HCAI and CAI.
Results: Among 422 patients, 129 patients were *S.aureus* BSIs and 293 *E.coli* BSIs. One hundred sixty five patients met criteria of NCI, 109 of HCAI, and 148 of CAI. HCAI was similar to nosocomial infection in terms of frequency of malignancy, predisposing factor, source of infections and in-hospital mortality. MRSA prevalence of HCAI was similar to that of NCI (43.8% versus 63.8%, $p > 0.05$) but was not similar to that of CAI (43.8% versus 7.1%, $p = 0.001$). But, *E.coli* resistance patterns of HCAI were similar to that of CAI (18.2% versus 14.2%, $p > 0.05$ in ampicillin/sulbactam, 7.8% versus 2.5%, $p > 0.05$ in cefotaxime, and 6.5% versus 2.5%, $p > 0.05$ in ESBL-producer) but were not similar to that of NCI (18.2% versus 38.5%, $p = 0.004$ in ampicillin/sulbactam, 7.8% versus 19.8%, $p = 0.026$ in cefotaxime, and 6.5% versus 16.7%, $p = 0.042$ in ESBL-producer). Prevalence of quinolone resistant *E.coli* was in order as follows; NCI (34.4%), HCAI (23.4%), and CAI (14.2%).
Conclusion: HCAI was similar to NCI in clinical characteristics and MRSA prevalence but not similar in resistance patterns of *E.coli*. The further consideration is needed for the definition of HCAI with regard to Gram-negative infections.

P1000

Secular trends of antimicrobial resistance of blood isolates in a newly founded Greek hospital

P. Rafailidis, S. Kasiakou, P. Morfou, G. Georgoulias, D. Nikita, M. Falagas *(Athens, GR)*

Objectives: Antimicrobial resistance is one of the most challenging issues in modern medicine. We sought to evaluate the secular trends of the relative frequency of blood isolates and of the pattern of their *in vitro* antimicrobial susceptibility in our hospital during the last four and a half years.
Methods: We focused on Gram-negative and Gram-positive bacterial isolates from cultures of blood specimens. Susceptibility testing that was performed with the Vitek 2 system, applying the criteria suggested by the Clinical and Laboratory Standards Institute (CLSI), followed isolation of bacteria. We compared the antimicrobial resistance of blood isolates of two periods: the first period was 1/1/2002-31/12/2003 (1200 isolates) and the second period 1/1/2004-30/6/2005 (774 isolates).
Results: Overall, the data regarding the relative frequency of blood isolates in our newly founded hospital do not differ significantly from those of hospitals that are functioning for a

much longer period of time. Out of 2156 bacterial isolates Coagulase negative staphylococci were the commonest blood isolates (52.5% of total). The relative frequency of other Gram-positive and Gram-negative microorganisms was the following, in descending order: *Escherichia coli* (8.9%), *Staphylococcus aureus* (5.9%), *Pseudomonas aeruginosa* (5.2%), *Klebsiella spp.* (4.8%), *Acinetobacter baumannii* (4.1%), and *Enterococcus faecalis* (2.2%). A noteworthy emerging problem is the increasing antimicrobial resistance of Gram-negative bacteria, mainly *Acinetobacter baumannii* and *Klebsiella pneumoniae* to various classes of antibiotics. *Acinetobacter baumannii* isolates showed an increase of resistance to amikacin ($p = 0.019$), ciprofloxacin ($p = 0.001$), imipenem ($p < 0.001$), and piperacillin/tazobactam ($p = 0.01$) between the first and second period of the study.
Conclusion: An alarming increase of antimicrobial resistance was noted during our study for Gram-negative bacteria, especially *Acinetobacter baumannii* and *Klebsiella pneumoniae.*

P1001

Hospital-acquired bacteraemia: occurrence of pathogens throughout a decade

H. Moraitou, M. Makarona, A. Koundourakis, I. Kousseris, N. Makrygiannis, V. Tzolos, M. Panagi, S. Kanavaki *(Athens, GR)*

Objectives: The isolation frequency and resistance profile of the commonest bacteraemia-related organisms were retrospectively studied throughout the periods 1991–1995 and 2001–2005, covering a decade, in 'Sotiria' Chest Diseases Hospital of Athens.
Methods: All bacteraemias were considered as nosocomial, since they occurred at least 48 hours after patient's admission to hospital. The cases were analysed in relation to responsible organisms and their antibiotic resistance profiles. In our Hospital, in Period A (1991–1995), 426 patients developed a nosocomial bacteraemia, while in Period B (2001–2005) 1872.
Results: In periods A and B, the isolation frequency of Gram (+) organisms was respectively for *S. aureus* 8.2% and 15.5%, *S. epidermidis* 27.5% and 24.0%, CNS 2.1% and 12.3%, *S. pneumoniae* 3.5% and 1.8%, *E. faecalis* 3.5% and 3.2%, *E. faecium* 0% and 1.6%, *K. pneumoniae* 4.9% and 6.5%, *E. coli* 5.4% and 9.7%, *P. aeruginosa* 5.2% and 8.1%, *A. baumanii* 1.9% and 3.9% and *S. maltophilia* 0.24% and 6.3%, respectively.The main characteristics in antibiotic profiles in periods A and B, included: MRSA occurrence 69.0% and 31.1%, oxacillin-resistant *S. pneumoniae* 0% and 8.8%, GRE faecalis 0% and 1.6%, and GRE faecium 0% and 13.3%, ESBL-producing *K. pneumoniae* 0% and 46.7%, ESBL-producing *E. coli* 0% and 1.1%, MBL-producing *K. pneumoniae* 0% and 23.7%, carbapenem-resistant *P. aeruginosa* 0% and 30.2% and carbapenem-resistant *A. baumanii* 0% and 58.1% respectively.
Conclusions: It is worthy of remark:
- The excessive rise in bacteraemia cases during period B (2001–2005), which could be partly attributed to the recent development of Oncology Unit, as well as University Internal Medicine Unit in our Hospital
- The decreasing frequency of MRSA isolations, probably due to control measures taken
- The increased incidence of oxacillin-resistant *S. pneumoniae*
- The recent occurrence of glycopeptide-resistant enterococci
- The emergence of highly resistant organisms, such as ESBL-producing *Enterobacteriaceae*, MBL-producing *K. pneumoniae*, and carbapenem-resistant *P. aeruginosa* and *A. baumanii*, and,
- The striking incidence of *S. maltophilia*, which is now considered as a serious nosocomial pathogen.

P1002

Positive central venous catheter cultures and bloodstream infections

C. Papadopoulou, Z. Afkou, G. Kythreotou, D. Tokatlidou, M. Tsivitanidou, D. Sofianou *(Thessaloniki, GR)*

Objectives: The purpose of this study was to determine the spectrum of microorganisms isolated from positive central venous catheter (CVC) cultures and to assess the correlation between bacteremia and positive CVC cultures.
Methods: From January 2002 to July 2005, a total of 1737 CVC sent for microbiological analysis were studied. Catheter tips were considered as infected when they yielded equal or more than 15 colonies using the semi quantitative Maki's method. Blood cultures were performed using the BacT/Alert automated system. Identification of microorganisms was performed using the VITEK 2 automated system (bioMerieux).
Results: Among the 1737 CVC cultures, only 287 (16.5%)were considered as positive. The microbial agents isolated from the positive CVC cultures were: *Staphylococcus epidermidis* 85 (29.5%), *Acinetobacter baumannii* 62 (21.5%), *Pseudomonas aeruginosa* 34 (11.85%), *Klebsiella pneumoniae* 21 (7.3%), coagulase-negative staphylococci 40 (13.9%), *Enterococcus faecalis* 14 (4.9%), *Staphylococcus aureus* 10 (3.5%) and other gram negative bacteria of the genus of *Enterobacteriaceae* 21 (7.55%). Furthermore, 97 positive blood cultures drawn from peripheral vein in patients with positive CVC cultures were studied, in order to evaluate if the CVC was the source of bloodstream infection. In 45 cases (46.4%)the same microorganisms were isolated in both CVC and blood cultures demonstrating that catheter, previous colonized by microorganisms of skin flora as well as nosocomial pathogens was the entry site of bacteremia. The most common microorganisms recovered from blood cultures were the following: *A. baumannii* 14 (31.1%), *S. epidermidis* 8 (17.7%), *P. aeruginosa* 7 (15.6%), *K.pneumoniae* 7 (15.6%), *E. faecalis* 2 (4.4%) and other *Enterobacteriaceae* 7 (15.6%).
Conclusions: (i) *S.epidermidis* were mainly responsible for catheter-related infections in our hospital.(ii) Catheter-related bacteremia was considered in only 46.4%.(iii) Aseptic techniques as well as continuous microbiological surveillance studies leading to effective empiric treatment of catheter-related infections are required.

P1003

Laboratory-based epidemiology of nosocomial candidaemia in a tertiary teaching hospital

L.G.O. Cardoso, P. Trabasso *(Campinas, BR)*

Objectives: To describe the epidemiology of nosocomial candidemia in a 400-bed teaching hospital.
Methods: Prospective follow of all patients with positive blood cultures for *Candida* species was done between August-2003 and October-2004. Searching for underlying diseases, presence of central venous lines, use of antibiotics and antifungal drugs prior to candidemia, and treatment were performed. Outcomes were survival thirty days after candidemia or death.
Results: Seventy-three patients with candidemia were followed (65.8% male), mean of age was 47.3 years (range 0 to 87). Previous episodes of bacteremia occurred in 31.5%. Isolation of *Candida* in other sites before candidemia was present in 15.1% of patients. The most frequent underlying disease was cancer, diagnosed in 35.6%. Diabetes mellitus, cardiopathy, nephropathy, pulmonary or neurological diseases were presents in less than 20% of cases. Almost half of patients (49.3%) were submitted to surgery in the last three months. Antifungal prophylaxis with fluconazol was received by 13.7%.

Antibiotics for more than 24 hours were previously used in 97.2% of cases. At the moment of candidemia, mechanical ventilation was present in 30.1% of patients, parenteral nutrition in 57.5% and central venous lines in 83.6%. Hemodyalisis in the 72 hours before candidemia were performed in 13.7% of cases. From all *Candida* isolates, 53.4% were *C. albicans* and 46.6% *C. non-albicans* (23.3% *C. tropicalis*, 15.1% *C. parapsilosis* and 8.2% *C. glabrata*). None risk factor or underlying disease was statistically related with any species of *Candida*. Antifungal therapy for more than 72 hours was given to 64.4% of patients. Overall mortality in thirty days was 47.9%. Antifungal treatment for more than 14 days was the only variable associated with lower mortality. No difference in the mortality rate was detected between different *Candida* species infections.
Conclusion: Candidemia is a frequent and serious nosocomial infection, with high mortality. Frequency of non-albicans species of *Candida* is high, but identification of species with lower susceptibility to fluconazol was not common. Antifungal treatment for more than 14 days was associated with lower mortality. Death in the first 72 hours of treatment was not rare and reinforces the importance of suspicion of candidemia in risk patients and early start of therapy.

P1004

Nosocomial bloodstream infection following cardiac surgery in children

N. Jashiashvili, A. Nanuashvili, M. Chkhaidze *(Tbilisi, GE)*

Objectives: Hospital infections continue to be a major problem, causing high morbidity and mortality in surgical clinics and significantly increasing the length of hospitalization and cost of treatment. Nosocomial bloodstream infection have repeatedly identified as the most frequent type of nosocomial infections in pediatric patients. Although, it is known that the frequency of infectious complications, as well as their etiology is rather different in various geographical regions, sometimes even at the concrete hospitals.The aim of our study was to determine the frequency and etiology of hospital bloodstream infection following cardiac surgery in children and to find out whether the emergency operation increases the frequency of bloodstream infection or not.
Methods: We studied 421 patients at Paediatric Cardiac Surgery Clinic, who were operated in the period from January 2001 till December 2004. The age of the patients varied from 1 day to 18 years. In 383 cases the operations were planed and in 38 cases-emergency.
Results: Out of 421 patients hospital infections occurred in 66 (15.7%) patient. The frequencies of infectious complications were as follows: pneumonia −32 (7.6%), bloodstream infection −14 (3.3%), wound infection −10 (2.4%), urinary tract infection −7 (1.7%), endocarditic −2 (0.5%), pericarditis −1 (0.2%), non differentiated site infection −12 (2.9%). The most cases of bloodstream infections occurred in children < 1 year of age. Bloodstream infection in 6 cases were caused by Gram-negative bacteria: *Klebsiella pneumoniae* −3, *Serratia marcescens* −2, *Acinetobacter baumanni* -1 and in 8 cases by Gram-positive bacteria: *Staphilococcus epidermidis* -7, *Streptococcus salivaris* -1. The rate of bloodstream infections following 383 planed operations was 3.4% and after the 38 emergency operations-2.6%.
Conclusion: The bloodstream infection was the second most frequent infectious complication following cardiac surgery in children. In the etiology of bloodstream infection the most frequent gram-positive bacterium was *Staphilococcus epidermidis* and the most frequent gram-negative bacteria-*Enterobacteriaceae*. The frequency of bloodstream infection was not significant different between emergency (2.6%) and planed operations (3.4%).

P1005

Nosocomial bloodstream infections in the cardiovascular surgery centre, Iasi, Romania

A. Mereuta, M. Dan, L. Colar, G. Tinica, G.I.M. Georgescu, D. Buiuc *(Iasi, RO)*

Objectives: To analyse the aetiology, the origin, epidemiological features and outcome of nosocomial bloodstream infections (NBSI) in patients that underwent cardiovascular surgery.
Methods: A retrospective study of bloodstream infections was conducted between April 2001 and September 2005 in the Cardiovascular Surgery Centre of Iasi.
Results: There were 57 episodes of nosocomial bloodstream infections at 2068 admissions. Bloodstream infections were secondary to an infectious body site (lower respiratory tract, wound, urinary tract) in 35.08% of the episodes, catheter-related in 22.80% and in 42.10% of cases they had an unknown origin. Cell-wall deficient forms (45.61%) were the most prevalent microorganisms (26 strains, out of which 12 reverted to the classical state: 4 gram-positive cocci, 6 gram-positive bacilli, 1 Gram-negative bacilli, 1 anaerobe and 14 were L-stable), followed by *Staphylococcus aureus* and coagulase-negative staphylococci 12 strains (21.05%), gram-positive bacilli 11 (19.29%), fungi 8 (14.03%), HACEK group, gram-negative bacilli, *Enterococcus* spp. 3 strains each (5.26%), *Streptococcus pneumoniae* 2 (3.50%), anaerobes 1 (1.75%). The proportion of polymicrobial episodes was 3.5% (*Staphylococcus aureus* + *Haemophilus paraphrophilus* and *H. parainfluenzae* + *Enterococcus faecalis*) and fatality was 26.3%.
Conclusions: We found a high proportion of cell wall deficient forms, rarely reported before. This may be due to: blood culture collection under antimicrobial therapy (P = 0.016), especially beta-lactams; more adequate methods for blood culture processing: sucrose enriched media (Hemoline Performance Duo, bioMerieux, France) and acridine orange stain for microscopy of the blood culture.

P1006

Rifampin and minocycline impregnated central venous catheters: a meta-analysis of randomised controlled trials assessing catheter colonisation and catheter-related bloodstream infection

M. Falagas, K. Fragoulis, I. Bliziotis, I. Chatzinikolaou *(Athens, GR)*

Objectives: The use of antimicrobial-impregnated central venous catheters (CVC) for the prevention of CVC microbial colonization and of catheter-related bloodstream infection (CRBSI) is a controversial subject. We performed a meta-analysis of randomised controlled trials (RCT) of CVCs coated with rifampin-based antimicrobial combinations in order to further evaluate this subject.
Methods: The assessed primary outcomes were microbial colonization of the CVC and CRBSI. The analysed secondary outcomes were the occurrence of adverse events, emergence of resistant organisms and all cause mortality. Our main analysis included RCTs that compared the efficacy of CVCs impregnated with rifampin and minocycline to that of non-impregnated with antibiotics CVCs.
Results: 8 RCTs fulfilled our inclusion criteria, comparing 1262 CVCs impregnated with rifampin-based antimicrobial combinations to 1236 non-impregnated with antibiotics CVCs. Our primary analysis showed that rifampin-minocycline impregnated CVCs were associated with lesser rates of

colonization (OR 0.41, 95% CI 0.25–0.68) and CRBSI (OR 0.21, 95% CI 0.10–0.43). Sub analyses of studies comparing rifampin-minocycline impregnated CVCs to non-tunnelled, non-antimicrobial-impregnated CVCs (5 studies) demonstrated superiority of the impregnated catheters in preventing colonization (OR 0.35, 95% CI 0.24–0.52) and CRBSI (OR 0.21, 95% CI 0.10–0.43). Further analysis, of RCTs comparing CVCs impregnated with any rifampin-based combination of antimicrobials to non-impregnated with antibiotics CVCs (all 8 RCTs), demonstrated superiority of the rifampin-based combinations in reducing colonization (OR 0.33, 95% CI 0.19–0.58), and CRBSI (OR 0.17, 95% CI 0.09–0.32). None of the RCTs reported significant increase in mortality, toxicity, or emergence of resistance in any of the studied groups.
Conclusion: Rifampin-minocycline impregnated CVC are safe and effective in reducing the rate of CVC colonization and CRBSI. Additional RCTs are needed to provide more information on cost –analysis, on the possibility of emergence of microbial resistance, to evaluate more their benefit in long-term catheterisation and to compare them versus the new generation of CHSS – coated CVC.

P1007

Infections related with tunelled haemodialysis catheter

T. Aldabó, R. Luque, M.J. Marcos, Y. Blanco, J. Ibeas, E. Bouza for the Red Española de Investigación en Patología Infecciosa *(REIPI)*

Introduction: In Spain, every year 12000 CVC are inserted in patients on HD, 40% of them tunnelled (TC). Infection is the second leading cause of death in this population, and catheter-related bacteremia (CRB) a major cause of hospitalisation.
Objectives: 1) To asses the incidence, epidemiology and risk factors for CRB in patients with TC on HD. 2) To know the length of TC use (catheter survival).
Patients and methods: We prospectively analysed all consecutive TCs inserted in four Spanish hospitals from Sep/04 to Oct/05. We used IDSA definitions for intravascular catheter-related infections (CID 2001). Characteristics of patients and TC, and risk factor associated with CRB during insertion and removal, were analysed. All patients were followed until CRB, death, removal of TC or time for the end of the study (one year). Data were colleted by an ''on-line'' database system. Quantitative variable were expressed as median (range). Chi-square and T-student tests were used for statistic analysis.
Results: One hundred and twenty-eight TCs were inserted in 117 patients (57% males). Age: 71 years (20–88). Previous time on HD before TC insertion: 78.5 days (5–8701). Catheter survival: 162 days (2–366). Tunnelled catheters were used after other vascular accesses in 64% of patients. Six episodes of CRB were observed, with an incidence of 0.29/1000 catheter-days (total time 20711 catheter-days). The causative bacteria were: *E. faecalis* (N = 3), *S. aureus* MS (N = 2) and *S. aureus* MR (N = 1). Three tunnel infections were reported and two of them developed CRB. Time since catheter insertion to the onset of CRB: 23 days (5–141). Thirty-two TC were removed (although only 4 for CRB). Insertion in femoral or subclavian veins (RR 19, 95% CI 3–114; p 0.000), previous hospitalisation (RR 5.7, 95% CI 0.8–39; p 0.057) and surgical procedure (RR 13, 95% CI 0,9–178; p 0.022) 1-month before were associated with CRB. In the outcome we registered one death, one relapse (who later died) and two metastatic infections (septic arthritis and vertebral osteomyelitis). Two deads (33%) were CRB-related and another 3 deads (2.70%) were observed in the non-CRB group in one-year follow-up (RR 11.2, 95% CI 2.4–44.7).
Conclusions: The incidence of CRB with TCs was very low, however its mortality-related is high. Risk factors associated with CBR were non-jugular vein insertion, previous hospitalisation and surgical procedure performed until 1-month before.

P1008

Bacillus spp. bacteraemia in children on parenteral nutrition

E. Lebessi, G. Antonaki, A. Zafiropoulou, K. Malliou, N. Paleologou, M. Foustoukou *(Athens, GR)*

Objectives: To study the epidemiologic and clinical data concerning bacteremia due to *Bacillus* spp. associated with total parenteral nutrition (TPN), during a four-year period (2002–2005).
Methods: Patients, who presented clinical signs of bacteremia while receiving TPN, with blood and/or TPN solution culture positive for *Bacillus* spp. were included. Blood drawn from peripheral veins and samples of the discontinued TPN solutions were cultured by the BacT/Alert system (bioMerieux, France). TPN solutions were also cultured quantitatively. Identification of microorganisms was carried out using Gram stain, colony morphology, motility test and lecithinase production at egg yolk agar (Oxoid, England), BBL Crystal Positive ID kit (Becton Dickinson, USA) and/or API20E/50CHB (bioMerieux, France). Three epidemiological environment surveys in TPN preparation room (PPR) were performed, according to the hospital infection control guidelines, when clusters of episodes occurred (Sept 2003, Jun 2004, Oct 2005).
Results: During the study period, 23 episodes occurred in 20 patients ranged in age from 6 days to 14 years. Bacteremia due to *Bacillus* spp. was documented by positive blood culture in 14 cases. TPN was discontinued in 7 cases and TPN solutions were found to be contaminated with the same organism. *Bacillus* spp. was also isolated from TPN solutions involved in 9 episodes, with negative (n = 5) or not performed (n = 4) blood culture. *B. cereus* (11 strains) and *B. megaterium* (10 strains) were the most common species isolated, followed by *B. pumilus* (1 strain) and *Brevibacillus brevis* (1 strain). The implicated organisms were also recovered from the laminar flow-hood of PPR. Abrupt onset, presented with fever and/or chills, occurred in all patients, accompanied with vomiting and diarrhoea (one case) and thrombophlebitis (one case). Dramatic clinical response was observed to discontinuing the infusion. No deaths were observed.
Conclusions: The isolation of *Bacillus* spp. from the laminar flow-hood and the TPN solutions suggested the in-use contamination during the preparation and compounding of admixtures. *Bacillus* spp. due to spores formation can resist to disinfectants. We emphasize the need for stringent asepsis in the hospital pharmacy, in order to avoid the contamination of infusates with potential pathogens, widely distributed in the environment, such as *Bacillus* spp.

P1009

Survey of virulence factors of clinical isolates from neutropenic patients with enterococcal bacteraemia

S.H. Park, S-M. Choi, D-G. Lee, J-H. Choi, J-H. Yoo, W-S. Shin *(Seoul, KR)*

Objective: This study was aimed to survey the prevalence of virulence factors in enterococci from clinical isolates, relatedness with vancomycin resistance, and their impact on clinical outcomes, and furthermore to elucidate their role in pathogenesis of blood stream infection of enterococci at the Catholic HSCT center

Method: A Collection of Enterococcus isolates from blood in neutropenic patients with hematologic diseases between January 2000 and December 2004 and vancomycin resistant enterococci (VRE) isolated from rectum from the same patients at the time of VRE bacteremia were screened for the presence of virulence factor genes, such as those for cytolysin (cylA), gelatinase (gelE), aggregation substance (asa1), enterococcal surface protein (esp), hyluronidase (hyl) by PCR. PFGE of the isolates from blood and rectum of the same patients were done to evaluate their genetic relatedness.
Result: We collected 56 isolates from blood and 13 isolates from rectum of 11 patients at the time of VRE bateremia. Among the isolates from blood, *E. faecium* accounted for 80.4% (n = 45) and *E. faecalis* for 19.6% (n = 11). The proportion of vancomycin resistant isolates was 76.5% (n = 34) in *E. faecium* and 27.3% (n = 3) in *E. faecalis*. The esp gene and hyl gene were detected in 35 (77.8%) and in 12 (26.7%) of isolates of *E. faecium* and the genes asa1, gelE, and cylA were not detected among them. Of 11 strains of *E. faecalis*, 81.8% (n = 9) were positive for esp., 36.4% (n = 4) positive for gelE, 81.8% (n = 9) for asa1, 54.5 (n = 6) for cylA and 9.1% (n = 1) for hyl. None of the virulence factors was associated with vancomycin resistance. None of the virulence factors directly influence on clinical outcome. Thirteen rectal isolates of VR *E. faecium* were all positive for esp., 69.2% (n = 9) positive for hyl. PFGE of 10 pairs of isolates from blood and rectum revealed that 7 pairs were genetically matched.
Conclusion: The virulence factors were not associated with vancomycin resitance and did not influence clinical outcomes. High relatedness between rectal and blood isolates and the higher prevalence of esp. and hyl in rectal isolates suggested that the intestinal tract could be an important focus of bacteremia in neutropenic patients and another factors other than esp. and hyl might be associated with invasion of *E. fae*cium into bloodstream. The role of virulence factors in *E. faecium* is needed to be elucidated in the future.

P1010

Accuracy of surveillance blood culture to identify patients with low response to treatment and high 30-days mortality risk in MRSA bacteraemia: comparison to a standard clinical and biological follow-up

E. Forestier, Y. Hansmann, V. Remy, M. Mosheni, N. Lefebvre, O. Lesens, D. Christmann *(Strasbourg, Clermont-Ferrand, FR)*

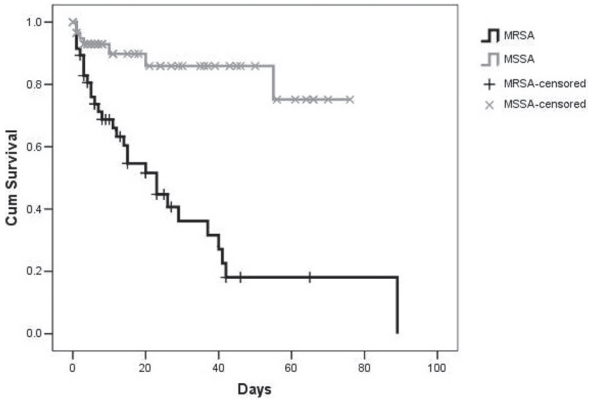
Objectives: Surveillance blood culture accuracy to evaluate treatment efficacy and predict death in patients with *Staphylococcus aureus* bacteremia remains unclear. Furthermore, whether it is more informative than a standard clinical and biological follow up is not determined. Finally, MSSA and MRSA bacteremia should be considered separately since differences in pharmacological properties of beta-lactamines and glycopeptides may influence the length of bacteremia.We evaluated the accuracy of positive surveillance blood culture to detect treatment failure and predict 30-days mortality in patients with MRSA bacteremia and compared it to a standard clinical and biological follow-up (fever, signs of sepsis, leukocytosis, C Reactive Protein level).
Methods: A prospective case-control study was realised between 1st October 2003 and 1st May 2005 in the Hôpitaux Universitaires de Strasbourg. A surveillance blood culture was systematically performed after 72 hours of presumed active antibiotic treatment and patients were followed for 30 days.
Results: Fifty patients presenting with MRSA bacteremia were included in the study. Eighteen (36%) patients had a positive surveillance blood culture. Main factor associated with breakthrough bacteremia was the presence of a central vascular device (CVD), particularly if it was considered as the portal of entry of the bacteremia ($p < 0.01$). Conversely patients treated with teicoplanine were less likely to have positive surveillance blood culture ($p < 0.05$) compared to those treated with vancomycin. Mortality rate in case of sustained bacteremia was 33.3% vs. 6.3% in case of non-sustained bacteremia ($p < 0.02$). Positive predictive value (PPV) for death of isolated breakthrough bacteremia was 33.3%. However, if considering only bacteremia developed from another portal of entry than a CVD, PPV increased to 91.7%.Aggravation of standard clinical and biological data after 72 hours of treatment was strongly correlated with 30-days mortality ($p < 0.01$) and its PPV (75%) and NPV (87.8%) for death were good, independently of any other factor.
Conclusion: Positive surveillance blood culture is a predictive factor for 30-days mortality in MRSA bacteremia but justifies treatment reconsideration only if the portal of entry is not a CVD. However, in most of cases, it appears not more informative than a standard clinical and biological follow-up.

P1011

Methicillin resistance as a risk factor for mortality in *Staphylococcus aureus* bacteraemia: an analysis from EARSS Enhanced Bacteraemia Surveillance in Ireland, 2005

A. Oza, S. Murchan, R. Cunney *on behalf of the Irish EARSS Steering Group*

Objectives: There is uncertainty in the literature about the contribution of methicillin resistance to mortality associated with *Staphylococcus aureus* blood-stream infection (BSI). We examined risk factors for mortality among Irish patients with *S. aureus* BSI.
Methods: A set of Irish hospital laboratories provide enhanced (clinical, demographic and risk factor) data on episodes of BSI reported to the European Antimicrobial Resistance Surveillance System (EARSS). Outcome data, including in-hospital mortality, has also been reported by a subset of laboratories since the beginning of 2005. We examined in-hospital mortality vs. survival before the end of each EARSS survey period (90 days) with logistic regression and survival analysis.
Results: There were 116 episodes of *S. aureus* BSI reported, with 39 in-hospital deaths, 71 discharged and 6 still in hospital at the end of the survey period. Of all the factors examined, length of stay (LOS) prior to BSI, age and meticillin resistance were all significant predictors of in-hospital mortality. Mortality among patients with MRSA BSI (n = 53, 60%) was significantly higher than among patients with MSSA BSI (n = 63, 11%). Cox regression, controlling for LOS and age, demonstrated that



ISSN: 1470-9465

methicillin resistance was the only significant contributor to mortality (HR 3.4, 95%CI 1.2–9.2). The baseline survival curve is shown.
Conclusion: Methicillin resistance remains a significant risk factor for in-hospital mortality among patients with *S. aureus* BSI after controlling for age and LOS.

P1012

Mortality from sepsis in Russia: identifying a gap in epidemiological data

D. Galkin, I. Gudkov, S. Zouzov, R. Kozlov *(Smolensk, RU)*

Objectives: Available epidemiological data on sepsis in Russia contain scarce clinical information and low reported mortality rates lead to underestimation of real figures. The study was designed to investigate incidence and clinical traits of sepsis according to autopsies' records in a large city in Central Russia for comparison with official statistics.
Methods: Autopsy protocols of all population of the city of Smolensk who deceased and undergone post mortem examination from the year 2000 to 2004 inclusive were assessed. Protocols where sepsis (as a post mortem diagnosis) was a major cause of death or a complication of an underlying disease were registered and processed using SAS version 8.2 software (SAS Institute, USA). That data were compared with reports of the Department of Health of the Smolensk Region.
Results: Overall 9346 autopsy protocols were evaluated. There were 116 autopsy records of patients who died with sepsis of whom 79 (68.1%) were men and 37 (31.9%) were women. Mean age was 42.5 ± 22.8 (0–90 years). All 116 (100%) septic patients died in a hospital setting. Length of hospitalisation until death was 11.7 ± 3.5 days and 1.9 ± 0.6 days took from death to post mortem evaluation. Sepsis as a primary diagnosis was recorded in 94 (81.1%) and as a complication of underlying conditions in 22 (18.9%) patients. Deviations with clinical diagnosis were noted in 24 (20.7%) of patients. Overall mortality rate by the autopsy records was 34.5 cases per 100,000 populations in comparison to 22.9 cases per 100 000 populations by the official data during the five-year study period.
Conclusion: Although the demonstrated difference in mortality rates from sepsis did not reach statistical difference ($p = 0.12$) it shows 1.5 superiority in incidence according to autopsy protocols in comparison with official data. This is possibly explained by lack of implementation of common definitions and inadequate disease registration recommendations in Russia. To close gap in the epidemiology data actions to reinforce use of unified sepsis diagnostic criteria are required at the nationwide level.

P1013

Cloxacillin sensitivity evolution in *Staphylococcus aureus* bacteraemias from 2001 to 2005 and their resistant phenotypes

M.M. Gallardo, M.V. García, R. Rodriguez, E. Granados, F. Ropero, A. Hernandez, A. Pinedo *(Málaga, ES)*

Objectives: To find the clinical and epidemiologic features associated to *S. aureus* bacteraemias and their resistant phenotypes.
Material and methods: Retrospective study of *S. aureus* bacteraemias that occurred from January 2001 to September 2005 in the Hospital in Málaga. *S. aureus* was isolated from 251 patients. The blood cultures were processed with BATEC-9240 system (Becton Dickinson). Antimicrobial suceptibilities were carried out by the MicroScan Walkaway automized system; resistance to cloxacillin was verified with saline MH with cloxacillin and E-test. The statistic study was carried out with SPSS 10.0.
Results: Of the 251 patients studied 68.1%were men and 31.9% women with an average age of 59.3 years old (14–89). In 40% of the cases were from the Medical Service, 24.7% from emergencies, 20.7% from the Intensive Care Unit and 13.9 %from the Surgical services. Average numbers of days in hospital were 29 days. In 93.6% of the cases it was a monomicrobian bacteraemia and in 62.2% the infection was of intrahospitalarian origin. Patient's progress was in 34.3% of the cases towards exitus. Average resistance to cloxacillin (CLO) during this period is 19.5%, even though its increase is clear during the years, going from 16.7% in 2001 to 22.7% in 2005. Resistance rates during the study period were 30.3% for erythromycin (E), 23.9% for ciprofloxacin (CI), 13% for clindamycin (CC), 11.2% for gentamicin (G) and 1.6%for rifampin (R). When resistance to CLO in relation to age was studied, an increase is found in the elderly age group (12.2% < 50 years old, 19.4% from 50–70 years old, 25.7% > 70 years old). An increase in resistance to CLO in intrahospitalarian strains (21.2%) was found compared to the extrahospitalarian (16.8%). When resistance to CLO was studied related to age, sex, hospitalisation days, hospital department, the authors haven't found significant differences. Resistance phenotype mostly found is CLO + CC (76.9%), followed by CLO + CI (73.2%), CLO + E (63.2%). Moreover, 42.9% strains showed CLO + CC + E and 38.77% CLO + CC + E + CI phenotype and 100% strains were sensitive to glycopeptides.
Conclusion: *S. aureus* bacteraemia is more frequent in men with an average age of 59 years old, who came from the medical department and of intrahospitalarian origin. Resistance to cloxacillin has increased in six points since 2001 (16.7%) to 2005 (22.7%). In 38.7% of the cases cloxacillin resistant strains are also resistant to clindamicin, eritromicin and ciprofloxacin.

ICU infections

P1014

Surveillance of hospital-acquired infections in intensive care units

J.L. Barrios, C. Ezpeleta, R. Blanco, M.J. Unzaga, J.A. Alava, R. Cisterna *(Bilbao, ES)*

Objective: The aim of this study is to know the rates of NI (Nosocomial Infections) in patients admitted into the ICU from January to June 2005 and to compare our results with those of the last NNIS report (National Nosocomial Infection Surveillance-October 2004).
Patients and Methods: The 1689 patients located in the Medical ICU and the 1385 patients admitted to the Coronary ICU (137 of them operated on open heart surgery) from January to June 2005 were included. All the patients were prospectively studied since the day they were admitted until the end of the episode by the Infection Control team. Variables under surveillance were the number of invasive devices: oropharingeal tubes, urinary

catheter and arterial and venous central catheters and site-specific infection rates associated to these devices by using as a denominator the number of patients at risk, patient-days, and device-days: Ventilator associated pneumonia (VAP), Urinary tract infections (UTI), Central line bacteremia (CLB), other secondary bacteremia (OSB) and primary bacteremia (PB), microorganisms, and antibiotic susceptibility, treatment and outcome. A computer based surveillance system (ENVIN-UCI) was used.

Results: 9 VAP, 6 UTI, 1 CLB, and 2 OSB were diagnosed in the Medical ICU and 9 VAP, 2 UTI, 1 CLB, 1 PB and 3 OSB in the Coronary ICU. Densities of incidence rates in Medical ICU were: VAP (11.43‰), UTI (3.86‰), SVCB (0.65‰) and total bacteremia (1.77‰). In Coronary ICU the values were: VAP (22.16‰)-6 VAP arrived in the postoperative period of open-heart surgery-, UTI (2.12‰), CLB (1.92‰) and total bacteremia (3.61‰). Microorganisms in Coronary ICU: Bacteremia: 2 *Candida* spp., 2 *S aureus*, 1 *S epidermidis*; UTI 2 *Candida* spp.; VAP 1 *S aureus*, 1 *S marcencens*. Microorganisms in Medical ICU: Bacteremia: 1 *Candida* spp., 1 *S aureus*, 1 *E coli*; UTI 2 *Candida* spp., 1 *E coli*, 1 *M morganii*, 1 *P aeruginosa*, 1 *K pneumoniae*; VAP 2 *S aureus*, 1 *P aeruginosa*, *S maltophilia*, *K oxytoca*, *H influenzae*.

Conclusions: Compared to the data of density of incidence for device-associated infections in the NNIS report, our results are in concordance except for VAP. In this case our data are higher than NNIS data, even more in our Coronary ICU. The rate in the coronary ICU without the post-operated patients is over the percentile 90 of the NNISS report (12.39‰). Even so the target for preventing VAP in the Coronary ICU of our hospital must be the group of patients undergoing open-heart surgery.

P1015

Infections in intensive care unit admitted multiple trauma patients: incidence, risk factors and mortality rate

Y-H. Choi, S-H. Baik, Y-J. Lee, K-S. Lee, Y-J. Oh, J-H. Park, S-S. Shin, K-J. Park, S-C. Hwang *(Suwon, KR)*

Objectives: Improvement in resuscitative managements of patients who sustained multiple traumas has increased survival rates but they still suffer from infections during the course of their recovery in the hospital. The purpose of this study was to survey the incidence and the site of infection and to define risk factors for infection and mortality in ICU admitted-multiple trauma patients.

Methods: Prospective record for APACHE II score day by day was conducted for all multiple trauma patients who were admitted at surgical ICU of Ajou university hospital from June 1. 2002 to May 30. 2004. Patients who were transferred from other hospitals after operation, or for more than 48 hours care or under the age of 18 were excluded. We reviewed medical records and all imaging studies retrospectively.

Results: A total of 108 patients (81 males) with mean age of 41 years (± 14 years) were included and their mean APACHE II score was 27.5 (± 18.9). Most common cause of trauma was blunt injuries (83 patients, 76.9 %) like traffic accidents and fall down. A total of 60 infections had occurred in 44 (40.7%) patients for an incidence of 12.5/1000 patient-days. The sites of infection were lower respiratory tract (22%), skin/soft tissue (11%), intra-abdomen (5.6%), central venous catheter (5.6%), urinary tract (2.8%), surgical site (2.8%) and others (11%). Mean onset time of infection was 25 days (± 41 days) and mean onset time according to infection sites were in order of following; intra-abdomen (3.8 ± 3.4 days), lower respiratory tract (15.8 ± 27.7 days), skin/soft tissue (17.3 ± 28.4 days), surgical site (23 ± 22.5 days), central venous catheter (41.7 ± 42.8 days) and urinary tract (50.0 ± 27.7 days). Mortality rate was 7.4%. Death was attributed to infection in four patients. In multivariated analysis, patients with infections are more likely to have prolonged hospital care (odds ratio (OR) = 1.042; $p \leq 0.001$), long-term ventilator care (OR = 1.556, $p < 0.001$) and open fractures (OR = 7.113, p = 0.018). APACHE II score (OR = 1.037, p = 0.044) and duration of mechanical ventilation (OR = 1.074, p = 0.001) were contributed to death, independently.

Conclusions: Multiple trauma patients have high risk of infection. Most common infection was lower respiratory tract infection. APACH II score was not a risk factor for infection but a risk factor for death.

P1016

Nosocomial infections in a neonatal intensive care unit: evaluation of frequency, risk factors and cost

I. Özgünes, H. Naz, N. Tekin, F. Aksit, E.D. Kartal, N. Erben, S.N. Alpat, G. Usluer *(Eskisehir, TR)*

Objectives: Nosocomial infections (NI) are important causes of morbidity and mortality in neonatal intensive care units (NICU). The objectives of this study were to identify the incidence and risk factors of NIs and cost of the NICU stay attributed to NIs in a Turkish NICU.

Methods: A prospective cohort study was conducted from November 1, 2004 to May 31, 2005, in the NICU of the Eskisehir Osmangazi University Hospital, 900-bed academic tertiary care centre with a 17–bed NICU. Exposure variables from maternal and newborn data were prospectively collected by daily visits. Diagnoses of NIs were based on the recommendations of the Centres for the Disease Control. Statistical analysis was performed with the software program SPSS (Version 10.0).

Results: Of the 309 patients surveyed, 48 (15.5%) had acquired NIs. The most common site of NIs was found as urinary tract (39.4%), eye-ear-nose-throat (18.8%), skin and soft tissue (18.8%) and blood stream (12.5%). The relationship between NIs and probable risk factors such as prolonged hospital stay (+ 7 days), low birth weight, previous antimicrobial usage, corticosteroid treatment, intubation, mechanical ventilation, CPAP, surgery, total parenteral nutrition, enteral nutrition and polycytemia were found statistically significant. The relationship between NIs and sex, gestational age, twin birth, route of delivery, home birth, low apgar scores were not statistically significant. Most common nosocomial pathogens were *Escherichia coli*, *Staphylococcus aureus*, *enterococci* and *Klebsiella* spp. The additional hospital stay because of NIs was 7.4 days. The median cost was found as 1061 Euro for newborns without NIs and 3082 Euro for newborns with NIs.

Conclusion: The risk factors for NIs detected in our NICU were similar to those defined worldwide. Gram-negative bacteria were the leading cause of NIs. The increase in the medical cost due to NIs was remarkable.

P1017

Colonisation surveillance can predict microbial aetiology of infection in the critically ill

F. Kontopidou, E. Papadomichelakis, A. Antoniadou, E. Koratzanis, E. Galani, A. Armaganidis, H. Giamarellou *(Athens, GR)*

Objectives: Appropriate empiric antimicrobial treatment directly affects infection mortality in the intensive care unit

(ICU). Microbial colonization of the respiratory and gastrointestinal tract (RT and GT) frequently precedes invasive infection. We retrospectively studied the ability of colonization surveillance to predict microbial etiology of subsequent infections.

Methods: The study was performed in a 5-bed medical-surgical ICU in a new university hospital from November 2003 to October 2005. Infection control policy included weekly surveillance cultures of bronchial secretion, urine and stool samples. We recorded all cases of ventilator-associated pneumonias (VAP) and bloodstream infections (BSI) based on data from patient files and the microbiology laboratory. The relationship between infectious etiology and most recent colonization was analysed, based on species, antimicrobial susceptibility patterns and molecular typing by REP-PCR of selected isolates.

Results: We recorded 21 VAP and 74 BSI cases (41 catheter-related). Pathogens isolated from VAP cases correlated with bronchial or stool colonizers in 83%. Prior RT colonization seemed most important. *Acinetobacter* sp. colonization of the RT predicted VAP etiology with a sensitivity of 69% and a specificity of 75%. Primary Gram (-) BSI pathogens were recent colonizers in 73% of cases, associated with both the GT and RT. *Klebsiella* sp. colonization of the GT predicted Gram (-) BSI etiology with a sensitivity of 67% and a specificity of 85%. In catheter-related isolates no relationship was observed between Gram (+) and prior colonization. However, 81% of Gram (-) previously colonized bronchial secretions or stool. REP-PCR techniques confirmed pathogen and colonizer concordance in all cases tested. Most cases of pathogen-colonizer discordance were due to either missed surveillance cultures or growth inhibition of sensitive strains in antimicrobial-containing media used for stool surveillance. Empiric antibiotic treatment based on colonization results permitted 90% adequacy in VAP and 80% in primary bacteremia treatment.

Conclusions: RT and GT colonization is strongly related to microbial etiology of subsequent infection. Systematic weekly colonization surveillance of RT and GT specimens could be helpful in implementing prompt and empiric antimicrobial therapy, especially for multi-drug resistant Gram (-) pathogens, in the ICU.

P1018

Administration of antibiotics via the respiratory tract for the prevention of ICU-acquired pneumonia: a meta-analysis of comparative trials

M. Falagas, I. Siempos, I. Bliziotis, K. Rellos, A. Michalopoulos *(Athens, GR)*

Objectives: The administration of prophylactic antibiotics via the respiratory tract is one of several strategies for the prevention of ICU-acquired pneumonia, an infection with considerable morbidity and mortality. We sought to systematically examine the available evidence regarding the effect of prophylactic antibiotics administered via the respiratory tract on the development of ICU-acquired pneumonia, mortality, colonization of the respiratory tract, emergence of antimicrobial resistance, and toxicity.

Methods: Relevant studies were identified by Pub Med search (9/1950 to 9/2005) and references from relevant articles. Trials that were included in the analysis provided comparative data regarding the above-mentioned outcomes. Two independent investigators performed the data extraction to calculate the effect of the studied intervention on clinically relevant outcomes.

Results: Eight comparative trials studying gentamicin (3 studies) polymyxins (3 studies), tobramycin (1 study), and ceftazidime (1 study) that studied 1,877 patients were included in our meta-analysis. ICU-acquired pneumonia (OR = 0.50, 95% CI 0.33–0.76) and colonization of the respiratory tract by *P. aeruginosa* (OR = 0.51, 95% CI 0.30–0.86) was less common in the group of patients that received the antibiotic prophylaxis. No difference in mortality was found between the compared groups (OR = 0.93, 95% CI 0.72–1.22). No serious drug-related toxicity was noted.

Conclusion: Prophylactic administration of antibiotics via the respiratory tract in patients in the ICU setting is associated with reduction of occurrence of ICU-acquired pneumonia. Despite the valid concerns for the development and dissemination of bacteria with antimicrobial resistance, the evidence supports the further investigation and consideration of this preventive strategy, at least in ICU patients at high risk for development of ICU-acquired pneumonia.

P1019

Serial evaluation of CPIS and C-reactive protein for diagnosing ventilator-associated pneumonia

M. Koeman, M.J.M. Bonten *(Utrecht, NL)*

Background: Diagnosing Ventilator-Associated Pneumonia (VAP) remains difficult without the existence of a clinically available gold standard. Generally, VAP is diagnosed upon a combination of clinical, radiological and bacteriological criteria, described in the Clinical Pulmonary Infection Score (CPIS) and many physicians include C-reactive Protein (CRP) in their clinical judgement. Importantly, temporary trends of these variables are frequently used in daily practice, though diagnostic properties of dynamic values of CPIS and CRP have not been compared to the diagnostic performance of their static values. We, therefore, aimed to compare diagnostic performance of static and dynamic values of CPIS and CRP in diagnosing VAP.

Methods: 385 consecutive patients, previously included in a randomised VAP-intervention trial, all needing mechanical ventilation, were studied. CPIS and CRP were obtained daily. Values determined at the day of diagnosis were considered static values. Dynamic values were defined as the difference between the day of diagnosis and 1 (d1) and 2 (d2) days before in patients developing VAP. In patients not-developing VAP, CPIS and CRP on any day were considered static and d1 and d2 were calculated for all possible sequential days. Reference diagnosis of VAP was based upon clinical judgement of responsible physicians with independent adjudication by 3 intensivists.

Results: 52 patients developed VAP. Static CPIS scores were 3.24 ± 2.29 on 2226 VAP-free patient days and 6.02 ± 1.50 on days of VAP diagnosis (Area Under the Curve (AUC) of Receiver Operating Characteristic (ROC) being 0.826, 95%CI: 0.783–0.870), with an optimal CPIS cut-off of 6 (sens 73%, spec 81%). No temporary trend in CPIS was discernable for patients developing and not-developing VAP, whereas CPIS scores clearly increased towards day of VAP diagnosis. Yet, AUC for d1 and d2 CPIS values were 0.786 (95%CI 0.715–0.857) and 0.766 (95%CI 0.694–0.838), comparable to static CPIS. Although static CRP was higher for patients with VAP (187 ± 117 versus 134 ± 139 mg/L; p = 0.024), the AUC of static CRP was 0.549 (95%CI 0.46–0.638) and diagnostic properties did not increase when using dynamic CRP values: AUC 0.484 (95%CI 0.394–0.574) and 0.486 (95%CI 0.399–0.572) for d1 and d2, resp.

Conclusions: Use of temporal trends in CPIS does not improve diagnostic performance. Neither static nor dynamic CRP-values appear to be reliable for VAP diagnosis.

P1020

Aetiology and timing of bloodstream infections in a liver intensive care unit

F. Pink, E. Sizer, W. Bernal, G. Auzinger, J. Wendon, I. Eltringham *(London, UK)*

Objectives: To determine the aetiology and timing of bloodstream infections on an adult liver intensive care unit and compare results with previously published data from the same Unit.
Methods: Retrospective study of 1074 adult patients admitted to a liver intensive care unit for more than 24 hours between January 2003 and July 2005. APACHE II and risk score on admission, clinical data relating to aetiology of liver failure, transplantation, mortality were collected in addition to blood culture organism identification and antimicrobial susceptibility. Standard criteria were adopted for definition of bacteraemia. Chi-square and Fisher's exact tests were used to determine associations between categorical variables.
Results: 209 patients (19.5%) had at one or more bacteraemia with a total of 403 isolates including 14 repeat episodes with the same species. The mean time from admission to the LICU to first significant bacteraemia was 10.9 days. 170 isolates were Gram positive, 206 Gram negative and 27 were yeasts. The median time from to first bacteraemia for different organisms was as follows; *Escherichia coli* and meticillin-sensitive *Staphylococcus aureus* (8.5 days) *Enterococcus faecalis* (9 days), Multi-resistant *Acinetobacter baumanii* (9.5 days), MRSA, coagulase-negative staphylococci, yeasts and *Enterococcus faecium* (11 days), *Klebsiella* sp. (12 days), *Stenotrophomonas maltophilia* (13.5 days), *Enterobacter* sp (19 days) and *Pseudomonas* sp (22.5 days). For a number of organisms this difference reached statistical significance. Mean APACHE II scores on admission were 21.2 those who developed a bacteraemia compared with 13.8 for those who did not.
Conclusions: Compared with earlier studies at the same centre these data suggest significant differences in the timing and sequence of organisms isolated from septic patients on our LICU. In this study infections with members of the *Enterobacteriaceae* and Gram-positive organisms appeared later. This may reflect changes in the prevalence of different species or strains and/or the impact of early pre-emptive use of antibiotics on the development of infection in liver failure patients. The significance of this in terms of management of sepsis is discussed.

P1021

Epidemiology of bloodstream infections in an Israeli general intensive care unit: a 6-year analysis

T. Mashiach, H. Sprecher, R. Finkelstein *(Haifa, IL)*

Objective: To evaluate the epidemiological features of nosocomial bloodstream infections (BSIs) observed at the General Intensive Care Unit (UTI) of our medical centre in the past 6 years.
Methods: Data were collected retrospectively from our computerized records from 1January 2000 through 31 October 2005. Potentially skin contaminants, including episodes caused by only one positive blood culture yielding coagulase-negative staphylococci were excluded. Multiple cultures of the same species were considered to be a single infection.
Results: During the study period there were 1321 episodes of BSIs. Aerobic gram-positive organisms accounted for 44% of cases, aerobic gram-negative organisms accounted for 46%, 4% were caused by fungi and 6% were caused by a diversity of other organisms, including anaerobes. The most common organisms were coagulase-negative staphylococci (26%), *Klebsiella* spp. (11.5%), *S. aureus* (10.2%) and *Acinetobacter* spp. (9.5%). Resistance rates for most pathogens increased gradually and strikingly during this period. From 2000 to 2005 the rate of MRSA increased from 59% to 77% (an increase of 30.5%), the rate of resistance of Klebsiella to 3rd generation cephalosporins increased from 42% to 70% (an increase of 66.6%), and resistances of Pseudomonas and Acinetobacter to imipenem increased from 20% to 37% (an increase of 85%) and from 40% to 78% (an increase of 95%), respectively. Multiple drug resistance among *Acinetobacter* spp. during the study period ranged from 80% to 100%. Surprisingly, rates of VRE remained low (mean: 2.2%, range 0%–6%)
Conclusions: These data may allow clinicians to better target empirical therapy for bloodstream infections in the intensive care unit as well as to understand the dangerous relationship between antibiotic use and resistance in this setting.

P1022

Pseudomonas aeruginosa infection in ICU patients: highlighting the silent epidemic

A. Agodi, M. Barchitta, L. Giaquinta, M.A. Romeo, C. Denaro *(Catania, IT)*

Objectives: In order to identify, assess and apply relevant evidence for better health care decision-making, our study was addressed to evaluate whether *Pseudomonas aeruginosa* associated nosocomial infections (NI) in intensive care units (ICUs) originate mainly from patients' endogenous flora or from exogenous cross-transmission, by determining: i) the occurrence of *P. aeruginosa* carriage on admission; ii) the ICU-acquired *P. aeruginosa* infection and colonisation rates, by site; iii) the impact of cross-transmission using molecular typing data of the involved microrganisms.
Methods: A six months active surveillance survey was performed at the ICU of an Italian Hospital, in accordance with the HELICS protocol. Carriage and colonization definitions were as described by Bertrand et al. (2001). Molecular typing was performed by macro restriction analysis of the SpeI-digested genomic DNA. Interpretation of genomic relatedness was performed using well-established criteria. The presence of two indistinguishable strains in two patients was considered one episode of cross-transmission.
Results: During the survey period a total of 123 patients were admitted to the ICU. The incidence of *P. aeruginosa* carriage and colonization/infection on admission was 1.6% and 2.4% patients, respectively. The ICU-acquired colonization rate was 29.4% patients and the incidence density was 16.1‰ patient-days. The ICU-acquired infection rate was 36.1% patients and the incidence density was 19.8‰ patient-days. ICU-acquired pneumonia (VAP) was confirmed to be the first *P. aeruginosa*-specific infection type (51.2%), followed by UTI and local CVC related infections (18.6% each). Nineteen distinct clones were identified by macro restriction analysis. The impact of *P. aeruginosa* cross-infection was estimated to be at least 47.1%, but this figure was higher when cross-colonization was included (59.5%), thus defining the preventable proportion of all *P. aeruginosa*-sustained cross-transmission episodes.
Conclusions: *P. aeruginosa* infection in ICUs has been suggested to represent the tip of an iceberg, whereas colonization reflects the submerged part. The epidemiological scenario depicted by our analysis shows those cross-transmission is an important

means of *P. aeruginosa* infection and highlight the occurrence of a silent epidemic mainly sustained by a multiresistant clone, that in the absence of epidemiologic screening would have remained the submerged part of the iceberg.

P1023

Meta-analyses of the impact of inappropriate antibiotic therapy on mortality in patients with ventilator-associated pneumonia and bloodstream infections

E. Gillespie, A. Patel, C. Coleman *(Hartford, US)*

Objectives: Several studies have found that initial treatment of ventilator-associated pneumonia (VAP) and blood stream infections (BSI) with inappropriate antimicrobial therapy is associated with higher rates of mortality, but additional studies have failed to confirm these findings. To provide a stronger quantitative basis for addressing these postulated associations, we conducted a series of meta-analyses of existing relevant studies.
Methods: Three investigators systematically searched databases from 1966-September 2005 and reviewed citations in relevant articles to identify studies that met the following inclusion criteria: (1) randomised or observational trials, (2) compare patients receiving appropriate antimicrobial therapy (defined as an antibiotic regimen with demonstrated *in vitro* activity against the identified bacterial species associated with infection) and inappropriate antimicrobial therapy in the setting of VAP or BSI and (3) report data on incidence of mortality. We conducted mortality analyses, both with and without adjustment for confounding factors. A random-effects model was utilized in all analyses.
Results: All studies included were observational in nature. A meta-analysis of VAP studies utilizing unadjusted mortality data (n = 8 studies) demonstrated inappropriate antimicrobial therapy significantly increased patients' odds of mortality [odds ratio (OR); 2.03 (95%CI 1.35–3.06); Q statistic p-value = 0.23]. Similar results were seen upon meta-analysis of adjusted mortality data (n = 9 studies) [OR; 2.00 (95%CI 1.32–3.05); Q statistic p-value = 0.06]. A meta-analysis of BSI studies utilizing unadjusted mortality data (n = 16 studies) demonstrated inappropriate antimicrobial therapy significantly increased patients' odds of mortality [OR; 2.29 (95%CI 1.88–2.78); Q statistic p-value = 0.0003]. Similar results were seen upon meta-analysis including adjusted mortality data (n = 16 studies) [OR; 2.11 (95%CI 1.53–2.92); Q statistic p-value < 0.0001]. Assessment of the funnel plots and Egger's weighted regression statistics (p-value > 0.34 for all) demonstrated a low probability of significant publication bias in the VAP and BSI analyses.
Conclusions: There appears to be an association between inappropriate antimicrobial therapy and higher mortality in patients with VAP and BSI, thus emphasizing the critical importance of early appropriate antimicrobial therapy.

P1024

Antibiotic resistance and mortality in patients with severe pneumonia

R. Erbes, M. Raffenberg, H. Mauch, S. Wagner, M. Schmidt-Ioanas, H. Lode *(Berlin, DE)*

Background: Nosocomial infections in patients admitted to intensive care unit (ICU) are frequently caused by potentially resistant pathogen (RP). The present study aimed 1) to determine the influence of antibiotic resistance on the outcome and 2) to identify the risk factors for developing an ICU-acquired pneumonia by RP.
Methods: All patients with severe pneumonia admitted to ICU between 1998 and 2004 were prospectively included in study. Clinical data, markers of infection (CRP, leukocytes, differential blood count, purulent secretions) and quantitative culture of the respiratory samples (bronchoalveolar lavage, protected specimen brush, endotracheal aspiration) and bacterial diagnostic by blood culture were recorded and considered for distinguishing between colonisation and infection. RP was defined as a pathogen with resistance against at least two major antibiotic groups.
Results: In 66 patients of a total of 162 patients (48 female, mean age 62 ± 15 years) with pneumonia a microbial growth in the respiratory secretions and in 17 (10.4%) patients a RP was noted. Furthermore in 20 patients (12.3%), a RP could be isolated during the ICU-stay The mortality rate in patients with evidence of RP was significantly higher in comparison to the patients with infection by susceptible pathogens (46% versus 21.5%, p = 0.006). Factors associated with an infection by RP were: mechanical ventilation (p = 0.01), renal failure (p = 0.01), sepsis (p = 0.01). The mean duration of treatment was significantly longer in patients with RP in comparison to the group with susceptible pathogens or with no microbial growth (34.6 ± 64.1 versus 18.1 ± 23.5 and, respectively, 6.5 ± 6.8 days, p < 0.001).
Conclusion: Patients with severe pneumonia by resistant pathogens have a significantly higher mortality rate. The factors associated with acquiring pneumonia by a resistant pathogen were mechanical ventilation, renal failure and sepsis.

P1025

Antimicrobial resistance patterns of Gram-negative bacteria isolated from intensive care unit in a Greek hospital

P. Sikalidou, S. Karampa, C. Papadopoulou, G. Kythreotou, D. Sofianou *(Thessaloniki, GR)*

Objectives: The purpose of our study was to determine the antimicrobial resistance of gram-negative rods causing infections in patients from intensive care unit (ICU) of a tertiary care hospital in northern Greece.
Methods: From January 2004 to December 2004 a total of 1803 clinical specimens from ICU patients were admitted to the laboratory. All specimens were inoculated onto routine culture media. The blood cultures were performed using the BacT/Alert 30 automated system. The infections were characterized as nosocomial according to CDC criteria. Identification of microorganisms and susceptibility testing were performed using the VITEK 2 automated system (bioMerieux France).
Results: From 1803 clinical specimens, 568 (31.4%) were positive cultures with one or multiple microorganisms. Respiratory tract infections were most frequent (28.7%), followed by bloodstream infections (26.4%), urinary tract infections (23.4%) and surgical site infections (10%). 464 (81.2%) gram-negative bacteria were responsible for the infections in ICU. *Acinetobacter baumannii* were the most frequently isolated Gram negative species (n = 196; 42.2%), followed by *Pseudomonas aeruginosa* (n = 177; 38%), *Klebsiella*

Pathogens	Resistance rates(%) to							
	AN	CTX	FOX	CAZ	CIP	P+T	IMP	GEM
A.baumannii	73	99	100	99	100	51	50	12
P.aeruginosa	77	100	100	78	80	16	19	80
Klebsiella spp.	52	92	92	92	55	19	3	92
E.coli	82	18	0	18	36	0	0	0
P. mirabilis	40	70	80	70	70	0	0	40
Ent.cloacae	0	43	71	43	14	14	0	0

AN:Amikacin,CTX :Cefotaxime,FOX:Cefoxitin,CAZ:Ceftazitime, CIP:Ciprofloxacin, P+T:Piperacillin/tazobactam,IMP:Imipenem,GEM:Gentamicin.

spp.(n = 64;13.8%), *Escherichia coli* (n = 11;2.4%) , *Proteus mirabilis* (n = 10;2.2%), *Enterobacter cloacae* (n = 7;1.5%).The resistance rates (%)of gram negative microorganisms are presented in the table. A total of 58 out of 63 of *Klebsiella* spp., 2 / 11 of *E. coli* and 7 / 10 of *P. mirabilis* were resistance to third generation cephalosporins demonstrating an extended spectrum beta lactamase (ESBL) positive phenotype.
Conclusion: *Acinetobacter baumannii* were the predominated pathogen followed by *P.aeruginosa*. High resistance rates were observed for all investigated drugs. ESBL production appeared to be a major mechanism of resistance to beta lactams. Imipenem appeared to be the most active agent against the majority of isolates.

P1026

Incidence of methicillin-resistant *S. aureus* in a prospective study in critical patients (2002–2004)

P. Palacián, A. Rezusta, Y. Martin, J. Castillo, M. Revillo *(Zaragoza, ES)*

Objective: To determine clinical features, risk factors and outcome of infection due to *S. aureus* in critical patients.
Methods: Study prospective for 3 years period (2002–2004) in Universitary Miguel Servet Hospital, 313 patients with isolation of *S. aureus* from the four units of critical care. The microbiological studies were carried following the standards protocols. Epidemiological, clinical characteristics and outcome were analysed in a posterior exhaustive study.
Results: For a three-year period, 313 patients with isolation of *S. aureus* were detected in our study, which represents 4.73% from the total of patients attended in the critical care units, and we haven't observed significant changes in our study period. The isolate rates of methicillin-resistant *S. aureus* and methicillin susceptible *S. aureus* were 35.46% (n = 111) and 64.64% (n = 202) respectively. Of all patients whose mean age was 56.73% (slightly superior in-patients with MRSA isolated: 58.85 versus 54.61) 75.08% (n = 235) were male and 24.92% (n = 78) were female. 234 patients were prospectively included in a more exhaustive study. Infection was defined as hospital acquired in 87.61% (n = 235) and community acquired in 12.40% (n = 29). The most frequent underlying disease were heart disease 25.21% (n = 59), trauma 24.78% (n = 48), infectious disease 14.10% (n = 33), neurological disease 13.24% (n = 31) and neoplasm 11.53% (n = 27). Risk factors detected were mechanical ventilation 77.78% (n = 182), venous central catheter 70.51% (n = 165), thoracic tube 39.31% (n = 92) and surgery 29.06% (n = 68). Mortality in-patients with MRSA isolated were 44.88% versus 55.12% in-patients with methicillin-susceptible *S. aureus*.
Conclusions: *S. aureus* is a commonly isolated in critical ill patients and the knowledge of epidemiological and clinical characteristics may allow to optimise its management, treatment to reduce morbidity and mortality in critical care units, so as prevent the increase of methicillin-resistant *S. aureus* isolates.

P1027

Screening for MRSA on nasal swabs at admittance at intensive care units

C. Pina-Vaz, R. Carneiro, M. Espinar, M. Machado-Vaz, A. Rodrigues *(Porto, PT)*

Objectives: *S. aureus* is a well documented nosocomial pathogen being responsible for serious systemic infections. It is recommended to perform the screening for MRSA at the admittance in intensive care units. The traditional search for MRSA involves the detection of *S. aureus* and then the evaluation of the susceptibility profile to oxacilin. This procedure takes at least 48 h, providing a relatively late answer to clinicians. CHROMagar MRSA, a chromogenic medium for isolation of specimens allowing direct differentiation of Methicillin resistant *S. aureus* by colony colour. We decided to compare traditional way for searching MRSA with the use of this cromogenic medium.
Material and methods: 145 nasal swabs were cultured both on Columbia 5% blood sheep agar (Difco) and on CHROMagar MRSA medium (BD), incubated for 24 h and 48 h. Colonies compatible with Staphylococcus on blood agar plate were submitted to confirmatory tests like catalase and coagulase test; if positive, a disk diffusion test was performed to evaluate susceptibility profile to oxaciline. On CHROMagar medium we looked for mauve colonies that clearly differentiated from other bacterial species, which result in blue or colourless colonies. The mauve colonies of methicillin resistant *S. aureus* were all later confirmed using classical methodology.
Results: 22 positive cases of nasal carriage of MRSA were detected using blood agar after 48 h; in a few of them take one more day was needed to isolate the colonies that were mixed with gram negative bacilli. Nine additional more positive cases were detected on CHROMagar MRSA medium (all confirmed by classic identification methods) after 24 h.
Conclusions: Regarding the detection of MRSA, CHROMagar MRSA culture showed a sensitive method, giving reliable and faster results, in comparison with conventional medium, thus allowing tremendous savings of labour and time.

P1028

Genotypic characterisation and epidemiologic analysis of vancomycin-resistant enterococci isolated from patients in intensive care unit

A. Lambiase, M. Del Pezzo, F. Rossano *(Naples, IT)*

In the past decade there has been a rapid rise in the number of serious nosocomial infections caused by enterococci resistant to multiple antibiotics. Enterococci with high-level gentamicin resistance became a major concern beginning in the 1980s. Subsequently, ampicillin-resistant enterococci, including both β-lactamase-producing and no-producing strains, have been isolated in many hospitals. In recent years, glycopeptide-resistant enterococci have been reported in both European and North American hospitals. In Italy, the problem of VRE (vancomycin-resistant-enterococci) is emerging and it presents therapeutic problems.
Objectives: The present study describes the molecular characterization of clinical isolates of VR-*Enterococcus faecium*, obtained from patients in an Intensive Care Unit (ICU) of Naples.
Materials and methods: Over 2-years period (January 2003–December 2004), 26 isolated of VR-*E. faecium* was examined (resistance to vancomycin and teicoplanin was determined by a standard dilution micro method in accordance with NCCLS guideline). All strains were obtained from several biological samples, including urine, blood, catheters, respiratory samples of ICU patients. Van genotypes (Van-A, Van-B, Van-C) were determined by using PCR. Therefore, isolates were typed by pulsed field gel electrophoresis (PFGE) with *Sma*I as restriction enzyme.
Results: Our study demonstrated a prevalence of genotype Van-A (20/26 strains; 77%); other strains were Van-B genotypes (23%). Analysis by PFGE and a comparison of *Sma*I banding profiles showed 25 isolated belonged to unique PFGE groups (group A; patterns A1–A14, different in two to three band, accorded with Tenover's criteria) and one strains belonged to

unique PFGE profile (strain B). The unique strain B and group A five strains were Van-B genotype.
Conclusions: Presence of VR-*E.faecium* was related to risk factors, as immunosuppression, organ recipient status, and exposure to antibiotics. Results of our study suggest that the intrahospital spread of VR-*E. faecium* can occur. Knowledge of their epidemiology is essential for the control of further spread. Use of CHEF electrophoresis might be helpful to clinicians to initiate effective infection control measures to contain the spread of this organism.

Gastro-intestinal and hepatic infections

P1029

Norovirus as a cause of acute gastroenteritis in Northern Greece: detection with IDEIA and RT-PCR

S. Mitka, A. Ifantidou, S. Sotiriadis, D. Karabaxoglou, A. Kansouzidou *(Thessaloniki, GR)*

Objectives: Noroviruses (NVs) are identified as an important cause of gastroenteritis in humans. These viruses are also the leading cause of food borne or waterborne outbreaks of infectious intestinal diseases. Genogroup II (GII) and I (GI) are associated with human infection. The virus is transmitted through contaminated food or water, directly from person to person or by contact with contaminated surfaces. The aim of this study was the investigation of NVs as cause of acute nonbacterial gastroenteritis in Northern Greece.
Methods: A total of 141 faecal specimens from patients (111 children and 30 adults, 79 males and 62 females) with acute gastroenteritis were studied. The major criterion for the choice of the samples was the initial exclusion of the bacterial nature of the disease. For the detection of NVs antigen in all faecal specimens an IDEIATM test (DakoCytomation) was performed. This test utilizes GI and GII specific monoclonal antibodies in a solid-phase sandwich enzyme immunoassay for the detection of GI and GII NVs. Specimens that were found to be positive with the IDEIATM test (N = 23) were examined for the presence of NVs RNA by RT-PCR using three different pairs of primers. The RNA extraction was performed by Qiagen spin columns technique (QIA amp RNA kit). The viral RNA from the specimens was amplified using two pairs of primers of the RNA polymerase gene (PCR A, PCR B) and one pair located near the 3′ end of the genome (PCR C).
Results: Faecal specimens from 23 patients (19/111 children, 4/30 adults) were found positive with IDEIATM test (13 for GI, 9 for GII and 1 for GI and GII) representing 16.3% of the total specimens. PCR A showed positive result in 20 out of 23 specimen, PCR B in 17 and PCR C in 11 (9 specimens were positive with all three PCR's, 8 specimens were positive with PCR A and B, 2 specimens were positive with PCR A and C and 1 specimen was positive only with PCR A).
Conclusion: The present findings showed that NVs are a frequent cause of acute nonbacterial gastroenteritis in Northern Greece. To our knowledge, this is the first reported study to have investigated the NVs as a cause of gastroenteritis in Greece. Results obtained suggest a seasonal distribution of the disease, whereas there are no significant differences among ages. PCR A was the most sensitive of the three PCR's applied.

P1030

Epidemiology and molecular analysis of norovirus outbreaks in Ireland

S. Kelly, B. Foley, S. Coughlan, L. Dunford, H. O'Neill, B. Smyth, P. McKeown, M. Lynch *(Dublin, Belfast, IE)*

This study set out to describe the epidemiology of norovirus outbreaks in the island of Ireland, over a one-year period. The study commenced on 01/10/04. Epidemiological data from outbreaks was collected in an electronic database, which was established for this project. A link for the sharing of epidemiological and virological data was established leading to an enhanced data set. Samples from outbreaks in the Republic of Ireland were sent to the National Virus Reference Laboratory in University College Dublin for confirmation of the diagnosis by RT-PCR (Reverse Transcription Polymerase Chain Reaction). Due to enhance surveillance during the study period there was a high rate of submission of samples. In the North of Ireland, samples were sent to the Regional Virus Reference Laboratory at the Royal Victoria Hospital in Belfast where a nested PCR was used for diagnosis of norovirus. Sequencing was carried out on the PCR products to determine the circulating strains of norovirus in Ireland. Over a one-year period, 153 norovirus outbreaks were reported in the Republic of Ireland. In the North of Ireland, over the same period, 73 outbreaks of norovirus infection were reported to the Regional Virus Reference Laboratory. Results so far indicate that the majority of reported outbreaks in the island of Ireland are associated with hospitals and residential institutions. In the Republic of Ireland the noroviruses associated with the majority of outbreaks were a new variant of Genogroup II.4, known as the JAM strain. A small number of noroviruses associated with outbreaks in the Republic of Ireland belonged to Genogroup II.2. The database will be used as a source of data for the Food borne Viruses in Europe Network. Safefood-the Food Safety Promotion Board funded this research.

P1031

Emergence of *Vibrio cholerae* O1 biotype EL Tor, serotype Inaba during last summer outbreak in Iran

M. Rahbar, R. Sabourian, M. Saremi, M. Abbasi, H. Masoumi Asl, M. Soroush *(Tehran, IR)*

Objectives: Cholera is an endemic disease in Iran. The aim of this study was to determine epidemiology and antimicrobial susceptibility patterns of *Vibrio cholerae* O1 biotype EL Tor serotype Inabe, which were isolated in recent outbreak in Iran.
Methods: Stool samples were collected from patients suspected to have cholera admitted to hospitals and clinics. Specimens examined by conventional bacteriological methods. All isolates were sent to cholera reference laboratory for confirmation, stereotyping and susceptibility testing. Antimicrobial susceptibility testing was performed by disk diffusion methods as recommended by NCCLS, The antimicrobial drugs included Ampicillin (AM), Ciprofloxacin (CI), Co-trimoxazole (SXT), Tetracycline (TC), Erythromycin (EM), Nalidixic acid (NA) and Furazolidone (F). The E-test MIC method used for detection of minimal inhibitory concentration (MIC) for co-trimoxazole, nalidixic acid and erythromycin.
Results: In total 1118 patients diagnosed clinically and laboratory confirmation to have cholera, disease reported from twenty-six provinces. The majority of cases were from Tehran Qum and Hamadan with 219, 190 and150 cases respectively. 50% of patients were between 15–34 years old. 53% of patients were

male and 47% female, 97.7 % of patients had Iranian nationality, 2.3% from Afghanistan and, 0.3% from Pakistan. 20% of patients were hospitalised and 80% were outpatients. Case fatality rate was 1%. 1104 isolates were Inaba serotype and only 14 cases were ogawa serotype. Our studies revealed that the origin of *Vibrio cholerae* was consumption raw vegetable that watered by swage. We also isolated *V. cholerae* from swages. All isolates were resistant to co-trimoxazole, nalidixic acid, furazolidone, and intermediate to chloramphenicole. All isolates were susceptible to tetracycline, ciprofloxacin, and erythromycin. MIC for cotrimoxazole and nalidixic acid were 256 μg/ml and 1.5 μg/ml for erythromycin. The antimicrobial results showed that all isolates had the same susceptibility patterns.
Conclusion: Our study reveals that in recent outbreak caused by *V. cholerae* EL Tor serotype Inaba. All isolates were resistant to co-trimoxazole, nalidix acid and furazolidone

P1032

National surveillance of *Campylobacter* infections and resistance in the Netherlands; an overview 2000–2004

B.C. van Hees, M.J. Veldman-Ariesen, B.M. de Jongh, M. Tersmette, W. van Pelt *(Nieuwegein, Bilthoven, NL)*

Objectives: *Campylobacter* is the most frequent cause of bacterial gastroenteritis worldwide. We describe epidemiological features of culture-proven *Campylobacter* infections in the Netherlands over the years 2000–2004.
Methods: Data from two ongoing projects for surveillance of infectious diseases in the Netherlands were used, covering 3- and 8 million inhabitants respectively. Incidence and resistance rates were analysed over time, by region, by level of urbanization, for seasonal variation and for recent travel history.
Results: The incidence of culture-proven *Campylobacter* infections showed an incidental decrease in the year 2003, in time related to an avian flu outbreak in poultry. Patients in age group 60 + were tested most frequently, but found positive in only 3%. Age group 15–29 years had the highest percentage of positive cultures (12%). The incidence of *Campylobacter* infections was highest in the southern part of the Netherlands; 55.7 per 100.000 in the south versus an average of 39.1 per 100.000 in the other parts of the Netherlands. The incidence was much lower in the rural than the urban areas. High stable rates of resistance were observed for fluoroquinolones (35%). Resistance to erythromycin was low but increasing over the years. Highest resistance rates to erythromycine were found in the south of the Netherlands. Resistance rates increased with increasing urbanization level. An inverse relation was observed between the incidence of infection (high in summer, low in winter) and resistance to both fluoroquinolones and macrolides (relatively high rates during winter). Resistance to predominantly fluoroquinolones was considerably higher in travel- related infections (54%), as compared to endemic ones (33%).
Conclusion: We found regional differences in incidence and resistance rates, both being highest in the south of the Netherlands. Furthermore we found an inverse relationship between resistance rates to fluoroquinolones and macrolides and the incidence of campylobacteriosis. An explanation could be that during winter months poultry is the most important source for *Campylobacter* infections, while during summer isolates from other sources associated with a low rate of fluoroquinolone resistance become more important. The high resistance rates to fluoroquinolones warrant reconsideration of its use as drug of first choice in the empiric treatment of presumed *Campylobacter* infections.

P1033

The epidemiology and clinical presentation of hydatid cyst disease in patients in Imam, Sina, Shariati and paediatric medical centre hospitals between 1372 and 1382

Z. Abdi Liae, A. Soudbakhsh, M. Solemani, K. Kiyani *(Tehran, IR)*

Background: Hydatid disease is a zeonotic disease and the most serious disease in human. It's an important hygienic problem in any area of the world. It's endemic in Iran, because it's possible for parasite to continue its life cycle. So it must be in the differential diagnosis list of any patient with cystic lesion.
Methods: We reviewed the epidemiology and clinical presentation of 300 patients that admitted and treated in the Imam, Sina, Shariati and Paediatric medical centre hospitals because of hydatic disease.
Results:

- The disease involves person in the 3rd and 4th decade.
- There is not any sex preference.
- Householder women are the most patient group.
- Most patient's live in the city.
- The most common involved organ is liver and then lung.
- In liver the right lobe is involved more than left lobe.
- In lung the left lower lobe is involved more than the others.
- Kidney, CNS, bone, heart, spleen are other organ that involved rarely.
- Laboratory changes are not meaningful but in some patients eosinophilia may be helpful for diagnosis.
- In our patient's and in this research CT scan was the most useful modality for diagnosis.
- The other helpful diagnosis options are hydatid serology (IFA)
- Surgery was therapeutic choice for most patients.
- Disease had same seasonal distribution and no time preference was seen.
- The infection of the cyst was the most common complication.

Conclusion: Hydatidosis has a low mortality rate but because of the chronicity of the disease it has a high morbidity rate. So it's important to diagnose it early and treat it as soon as possible. For this reason in any patient with symptoms and signs that rise or suspicion to space occupying lesion, hydatidosis must be one of the major disease we think about and rule it out or prove it with appropriate modality.

P1034

Echinococcus granulosus: lethal effect of low voltage direct electric current on hydatid cyst protoscoleces

R. Ghasemikhah, H. Ghasemikhah, M. Sharbatkhory *(Tehran, IR)*

Objectives: We have studied a small-scale method for killing hydatid cyst protoscoleces using low voltage direct electric current.
Methods: After collecting hydatid cysts from infected organs of slaughtered animals, protoscoleces were cultured in four different media: hydatid cyst fluid, RPMI, normal saline, and Tris buffer, respectively. Protoscoleces from each of the above media were then transferred to an electrolysis device through which different electric current densities were applied. For measuring the survival rate of protoscoleces, flame cell movement and eosin staining was used.
Results: The results show that the survival rate of protoscoleces in hydatid fluid was dependent on the electric current density and the time of the applied current. Current densities of

62.5 mA/cm^2 (11 V), 53.71 mA/cm^2 (10 V), and 18.18 mA/cm^2 (5 V) after 1, 2, and 3 min, respectively, killed all the parasites in the hydatid fluid.
Conclusion: However, a current density of 7 mA/cm^2 (9 V) in RPMI medium after 3 min was most effective.

P1035

Single and multiple pyogenic liver abscesses: aetiology, clinical course and treatment

F. Mateos, I. Terrancle, G. Peralta, L. Fuentes, J. Pérez-Arellano, F. Amoros, R. Serrano, M.P. Geijo, I. Tárraga, M. Rguez-Martín, B. Rguez-Martín, J. Blanch, E. Martínez-Alfaro for the Infectious Diseases Group of the Spanish Society for Internal Medicine *(SEMI)*

Objectives: The aim of the present study was to evaluate the differences in etiology, in clinical course and treatment of patients with single and multiple pyogenic liver abscesses (PLA)
Methods: Multicenter and retrospective review of the PLA diagnosticated in eight Spanish hospitals from January 1997 to December 2003. Cases were included if the abscess was confirmed by imaging as well as by either documentation of an organism recovered from the abscess site or resolution of symptoms and signs after treatment. Statistical analysis was performed with the SPSS software package.
Results: A total of 175 patients with PLA were managed: 117 of them presented a single abscess (SA) and 58 had multiple abscesses (MA). The median age was 66 ± 1.2 SEM, and males were the most affected (65%). Hepatomegaly was more frequent in MA ($p < 0.05$). Serum levels of alkaline phosphatase ($p = 0.05$) and the neutrophils count ($p = 0.007$) were significantly higher in patients with MA than in those with a SA. SA was usually larger than 6 cm, and MA were smaller than 6 cm. SA was located on the right side of the liver in 119 (68.4%) cases and MA were located on the right side in 28 cases (48%). SA had a cryptogenic origin in 48 cases (41.3%) and MA had a biliary origin in 27 cases (47%). The responsible microorganism was identified in 80 cases (68.3%) of the patients with SA and in 30 cases (52%) of the patients with MA, (OR 1.98; Chi-Square $p = 0.05$). *Eschericia coli* was the most common etiological agent identified in cultures of blood and abscess aspirates in PLA. Percutaneous drenaige and antibiotic therapy were the most used treatments in both types of abscesses. The abscess related morbidity rate was higher in MA (28, 48%). The overall mortality was higher in SA (8; 13.8%) than in MA (10; 8.6%).
Conclusions: These results suggest that MA had a biliary origin, showing hepatomegaly, neutrophillia and rise of the alkaline phosphatase as their clinical presentation most frequently than SA. Microbiological identification was higher in patients with MA than in patients with SA.

P1036

Comparison of pyogenic liver abscess caused by non-*Escherichia coli* and *E. coli*

F. Mateos, I. Terrancle, G. Peralta, L. Fuentes, R. Serrano, V. Navarro, M. Hernández, B. Roca, F. Marcos, I. Tárraga, A. Hernández-Belmonte, E. Martínez-Alfaro for the Infectious Diseases Group of the Spanish Society for Internal Medicine *(SEMI)*

Objectives: The aim of the present study was to compare pyogenic liver abscess (PLA) caused by non-*Escherichia coli* and *Escherichia coli*.
Methods: Multicenter and retrospective review of the PLA diagnosed and treated in eight Spanish Hospitals from January 1997 to December 2003. Cases were included if the abscess was confirmed by imaging as well as by either documentation of an organism recovered from the abscess site or resolution of symptoms and signs after treatment. Statistical analysis was performed with the SPSS software package.
Results: A total of 175 patients with PLA were managed. *Escherichia coli* was the species most commonly isolated in cultures of blood and abscess aspirates: abscesses caused by *Escherichia coli* accounted for 26% (43) of the cases in which an organism was recovered. There was no difference in the epidemiological characteristics, duration of fever after admission, initial laboratory value and duration of antibiotic therapy. Mean age and female gender were higher in *Escherichia coli* s PLA (RR 2.45, CI 95% = 1121 to 5384). The most commonly identified underlying cause was biliary disease. The recovery of *Escherichia coli* guided to biliary origin [(RR 4.331 (CI 95% = 1916 and 9789)]. When comparing the size of *Escherichia coli* versus other causes of PLA, *Escherichia coli* PLA was greater than non-*Escherichia coli* abscesses ($p = 0.008$), and difference of diameter average of 1858 cm (CI 95% = 0505 and 3212). They were both most frequently located on the right lobe. There was no correlation between the microbiological findings and the presence of a solitary or multiple abscesses. The therapeutic modality (percutaneous drenaige and antibiotic therapy) carried out in two groups was not significantly different, neither were hospital stay, and morbidity and mortality rates.
Conclusion: These results suggest that PLA cause by *Escherichia coli* comprise a disease of biliary origin, higher age, female gender. *Escherichia coli* PLA was greater than non-*Escherichia coli* abscesses

P1037

Diabetes mellitus as a risk and prognostic factor for pyogenic liver abscess in Denmark

R.W. Thomsen, P. Jepsen, H.T. Sørensen *(Aalborg, Aarhus, DK)*

Objectives: Pyogenic liver abscess (PLA) is a rare but life-threatening disease with rising incidence rates reported from the United States and Europe. In Taiwan, diabetes mellitus has emerged as an overwhelming risk factor for PLA, whereas epidemiologic studies on diabetes as a risk factor for PLA in Western populations are sparse.
Methods: We examined diabetes as a risk factor and prognostic factor for PLA in Denmark during a 26-year period, using a large nationwide data set based on administrative registries. We identified all patients with a diagnosis of PLA in the National Registry of Patients, and randomly selected fifty gender- and age-matched population controls for each case. We used conditional logistic regression to estimate odds ratios (ORs) for PLA according to diabetes, adjusted for medical and surgical risk factors. Among patients with PLA, we compared adjusted mortality odds ratios in diabetic and other patients.
Results: The study included 1448 patients with a first hospitalisation with PLA between 1977 and 2003. The median age (range) was 65 (0–97) years, and 54% were men. A total of 11.1% of the patients with PLA had diabetes recorded before or on the matching date, compared with 2.5% of control subjects. After adjustment for potential confounding factors, the OR for PLA in persons with diabetes was 4.0 (95% CI: 3.3–4.8). At 30 days after discharge, the mortality was 24.8% for diabetic patients and 18.0% for non-diabetic patients with PLA. The adjusted mortality odds ratio in diabetic patients was 1.3 (95% CI: 1.0–1.9).
Conclusion: In this nationwide study, we found a fourfold increased risk and a poorer outcome of PLA associated with diabetes mellitus.

P1038

Bacteraemia is a poor prognostic factor in spontaneous bacterial peritonitis

J.H. Cho, W.B. Park, H.B. Kim, N.J. Kim, M-D. Oh, K.W. Choe *(Seoul, KR)*

Objective: Spontaneous bacterial peritonitis (SBP) is a major complication of liver cirrhosis. There are few data about prognostic significance of bacteremia in patients with SBP. So we performed a retrospective study to determine the influence of bacteremia on the mortality of patients with SBP.
Methods: All patients with SBP, in whom the microorganism was isolated from ascites and/or blood, were identified by retrospective review of clinical and laboratory records in Seoul National University Hospital in Korea from January 2002 to December 2004. They were classified into the bacteremic group and non-bacteremic group. The underlying liver function was determined by MELD (Model for End-stage Liver disease) score. Microbiologic response rate, ascites polymorphonuclear leukocyte count reduction rate, and SBP-related mortality were compared between the two groups. To identify the independent risk factors of mortality, a multiple logistic regression model was used to control for the effects of confounding variables.
Results: A total of 203 patients were enrolled in this study. Among 203 patients, 116 (57.1%) were bacteremic, and 87 (42.9%) non-bacteremic. *Escherichia coli* was the most common etiologic organism, followed by *Klebsiella pneumoniae*. The distribution of etiologic organisms was not different between the two groups. Stratified MELD scores showed no significant difference between the two groups. Microbiologic response rate (64.7% versus 74.7%, $p > 0.05$), and ascites polymorphonuclear leukocyte count reduction rate (34.3% versus 43.3%, $p > 0.05$) were not different between bacteremic and non-bacteremic group. But SBP-related mortality of bacteremic groups was significantly higher than that of non-bacteremic group (31% versus 8%, $p < 0.001$).Bacteremia (OR, 5.8; 95% CI. 1.9–16.9; $p < 0.01$) and MELD score (OR, 2.4; 95% CI. 1.1–5.1; $p = 0.017$) were independent risk factors in multiple logistic regression analyses of SBP related mortality.
Conclusion: Bacteremia is an independent risk factor of mortality in SBP.

Respiratory tract infections

P1039

Aetiology of lower respiratory tract infections among primary care patients of family physicians in Silesian province in the 2000–2005 period

I. Szymczyk, W. Lukas, J. Skorupka, J. Józwiak *(Zabrze, PL)*

Objective: Etiological analysis of sputum culture regarding seasonal fluctuations in family practitioners' patients.
Methods: The study group included both primary care (207) and clinically diagnosed patients (50) who had their sputum examination ordered and then performed in Silesian Analytic Laboratories in the2000–05 period. All subjects were registered in the family practise or hospitalised in the Silesian area. The including criteria were active infections of lower respiratory tract. The intake, transport and final identification of the material complied with laboratory standards.
Results: Number of positive cultures of all sputum samples taken from both primary care and hospitalised patients accounted for 77% and 68%, respectively. The most common pathogens of primary care patients were, in order of prevalence (excluding *Streptococcus viridans*): *Moraxella catarrhalis* (57%), *E.coli* 21%, *Staph.aureus* 11%, *Klebs.pneumoniae* 10%, *Pseud. Aeruginosa 6%*, *Proteus mirabilis* 4%. In clinically diagnosed patients these were: *Moraxella catarrhalis* 33%, *Klebs. pneum*. 22%, *E coli* 19%, *Pseudomonas aeruginosa* 19%, *Staph.aureus* 11%, *Str*. spp. 11%, *Str. pneumonicae* 7%. As for any seasonal fluctuations among primary care patients the higher number of positive samples was noted in April and October (15%), the least in June, July and August (3%).The results of our detailed analysis of primary care samples showed the higher percentage of *Moraxella catarrhalis* strains in Dec. (77%) and Jan.(75%) and the least in May (20%). In the other months it ranged from 50 to 60%. The higher percentage of *E.coli* isolates (70%) was observed in May, while in Mar., Jun. and Dec. they were absent. As for *Staphylococcus aureus* the most frequent detection was stated in Mar. and Dec.27% and 31%, respectively), whereas they did not emerge in summer months (from June to Sep.). The higher emergence of *Pseudomonas aeruginosa* was observed in June (20%) while *Klebs. pneumoniae* isolates tested positively mainly during the spring and summer period (10–20%).
Conclusions: 1. Because of preference of empirical therapy in the diagnostic procedures, examination of the sputum is seldom ordered by family physicians. 2. *Moraxella catarrhalis* isolates were the most common pathogen isolated from material of subjects with lower respiratory tract infections and tended to be more prevalent in winter months. 3. Obtained data may be useful in family practise when selecting the optimal treatment for lower respiratory tract infections.

P1040

Chronic exposure to smoking is not a risk factor for community-acquired pneumonia in the elderly population

Y.K. Cho, B.J. Park, H. Kim, N-K. Choi, S-M. Lee *(Incheon, Seoul, KR)*

Objectives: The relationship between cigarette smoking and the risk of community-acquired pneumonia (CAP) in elderly people remains controversial. Age and gender matched case-control study to analyse the association between cigarette smoking and the risk of hospitalised CAP in elderly people was performed.
Methods: The eligible cohort (n = 15,049) is derived from Korean Elderly Pharmacoepidemiology Cohort (n = 46,113) that is population-based dynamic cohort. The cohort members were over 65 years of age, living in Busan Metropolitan City between 1993 and 1998, and beneficiaries of Korean Medical Insurance Corporation (KMIC). The information on smoking status, alcohol consumption, past-medical history, height, weight, performance status, and other daily life related variables at their age of 65 years were collected by self-administered mailed questionnaire. Potential cases (n = 260) of hospitalised CAP, were collected from the KMIC medical claims database between January 1, 1993 and December 31, 2000. The incident cases of hospitalised CAP (n = 83) were confirmed through the medical records. Four controls were matched to each case randomly using age, gender, and year of questionnaire survey (n = 332). Adjusted odds ratios (aOR) were calculated from conditional logistic regression analysis after adjusting for the confounding effect of smoking, alcohol consumption, body mass index (BMI), performance status, and underlying lung or cerebrovascular disease.

Results: The median age of case patients is 76 years and 33 of 83 (40%) patients are male. Underlying lung disease (aOR, 7.17; 95% CI, 2.78–18.50) and alcohol ex-drinker (aOR, 5.83; 95% CI, 1.36–24.95) by the age of 65 years was associated with higher risk of hospitalised CAP. Smoking, total amount of cigarette smoking, and duration of smoking by the age of 65 years were not significantly related to the risk of hospitalised CAP. The other factors, including performance status, BMI, other underlying diseases, were not associated with the risk of hospitalised CAP.
Conclusion: Underlying lung disease and alcohol ex-drinker by the age of 65 years can be the independent risk factors for hospitalised CAP in elderly people in later period. The chronic previous exposure to smoking, however, is not a risk factor of hospitalised CAP in the same people. These suggest chronic respiratory damage due to cigarette smoking might not influence on the occurrence of acute lower respiratory tract infection in the community environment.

P1041

Antibiotic resistance patterns among respiratory pathogens isolated during 2003–2005 at a Turkish tertiary hospital

Z. Gülay, H. Özkul *(Izmir, TR)*

Objective: To evaluate the current epidemiology of resistance among *Streptococcus pneumoniae* (Sp), *Haemophilus influenzae* (Hi) and *Moraxella catarrhalis* (Mc) in Izmir, Turkey.
Methods: Trends in resistance were investigated retrospectively in a total of 185 Sp, 169 Hi and 58 Mc, which were isolated between January 2003 and September 2005. Antibiotic susceptibility tests were performed according to the NCCLS (CLSI) recommendations.
Results and Conclusion: The overall rate of resistance to penicillin (P) was 31% among Sp isolates. Intermediate resistance, which was 29 % in 2003 and 28.3 in 2004, increased to 37.8% in 2005. A similar trend was also observed for high level P resistance (7, 6 and 14.3 % in 2003, 2004 and 2005 respectively). Although it can be as high as 44.4% among pen I/R isolates, overall resistance to erythromycin was 15.1% with minimal variation for different periods of time. MLSb tpe resistance was dominant among ER isolates as shown both by phenotypic tests and PZR. No resistance to levofloxacin was detected. All of the Mc isolates were susceptible to amoxy-clav (AMC), fluoroquinolones and cefaclor. A small increment in b-lactamase positivity was seen over the years (78% in 2003 to 93% in 2005). Hi isolates were also universally sensitive to 2nd and 3rd gen cephalosporins and AMC with b-lactamase production ranging from nil (2003) to 4.3 % (2005). On the other hand, trimetoprim-sulfamethoxazole (SXT) resistance was high among all species. There is still a low rate of resistance to commonly used antibiotics among respiratory pathogens but an increase in resistance to penicillin was noted in Sp and to SXT in all three species.

P1042

Differences between Bedouin and Jewish population in clinical characteristics of patients admitted with community-acquired pneumonia

V. Novack, L. Avnon, K. Riesenberg, F. Schlaeffer *(Beer Sheva, IL)*

Objectives: Ethnic groups have different inherent risk factors for contracting community-acquired pneumonia (CAP). In Southern Israel (population: 985,000) two ethnic groups live side by side: predominantly urban Jewish population and a population of Arab Bedouins (population: 144,000–14.3%) who are in social transition from being desert nomads to a settled lifestyle.The objective of the study was to evaluate the differences in characteristics and outcome of CAP between these two populations for patients hospitalised during one winter season.
Methods: We conducted a hospital based prospective observational study. Soroka University Medical Centre is a 1200 bed tertiary care hospital, which serves as the only regional hospital for Southern Israel. During a 4-months period we assessed clinical as well as demographic characteristics of all patients hospitalised with CAP.
Results: 262 patients were enrolled, of whom 58 (22.1%) were Bedouins. Bedouin patients were younger than the rest of the cohort (60.0 ± 20 vs. 66 ± 17 years, $p = 0.05$) and had lower rates of cardio-vascular diseases such as ischemic heart disease and cerebro-vascular disease (20.7% vs. 39.2%, $p = 0.02$). Bedouins had higher smoking rates (39.7% vs. 19.1%, $p = 0.001$), higher prevalence of COPD (31.0% vs. 9.3%, $p = 0.001$) and diabetes (41.4 vs. 25.0%, $p = 0.01$). There was no difference between pneumonia patients outcomes research team (PORT) scores of Bedouin and Jewish population at admission (median 83 points vs. 85 points, $p = 0.61$). Bedouin patients had lower rate of pre-hospitalisation antibiotics therapy compared to the rest (12.1% vs. 25.5%, $p = 0.03$). There were no differences in the length of hospitalisation (median 4 days, $p = 0.8$) or 30-day mortality rate between Bedouin and Jewish patients (3.4% vs. 8.8%, $p = 0.26$).
Conclusions: There were a number of differences in clinical characteristics between Bedouin and Jewish patients admitted to the hospital with CAP. Higher smoking and COPD rates in Bedouin patients may affect the microbiology of the CAP pathogens and this should be taken into consideration when therapeutic decisions are made. Despite socio-economic differences between the two ethnic groups, there was no difference in severity of CAP and clinical outcomes.

P1043

Prognostic impact of haematological and non-haematological malignancies on mortality of hospitalised pneumonia: a Danish population-based cohort study

M. Nørgaard, A. Riis, R.W. Thomsen, H.C. Schønheyder, H.T. Sørensen *(Aalborg, Aarhus, DK)*

Objectives: To examine mortality of hospitalised pneumonia within 90 days of follow-up among patients with haematological and non-haematological malignancies.
Methods: We conducted a population-based cohort study in three Danish counties (population 1.4 million). Through the regional hospital discharge registries we identified all adults (≥ 15 years) with a first-time hospital discharge diagnosis of pneumonia between 1999 and 2003 (n = 41 793). We furthermore obtained information on previous discharge diagnoses including haematological and non-haematological malignancies. The patients were followed for mortality through the Danish Civil Registration System. We estimated mortality ratio (MRR) using Cox regression analysis with adjustment for calendar year, age, gender, and co-morbidity.
Results: Among 41 793 patients hospitalised with pneumonia 423 (1%) had a haematological malignancy and 3138 (8%) had a non-haematological malignancy diagnosed prior to hospitalisation with pneumonia. Another 87 patients had both a haematological and a non-haematological malignancy. Cumulative 90-day mortality following pneumonia in patients without any malignancy was 21% vs. 31% in patients with haematological malignancies and 34% in patients with non-

haematological malignancies. Compared with patients without any malignancy the adjusted 90-day MRR was 1.6 (95% CI 1.4–2.0) for patients with haematological malignancies and 1.5 (95% CI 1.4–1.6) for patients with non-haematological malignancies.
Conclusion: Among patients with a first-time hospitalisation with pneumonia both haematological and non-haematological malignancies are associated with substantially increased 90-day mortality.

P1044

Outbreak of three related cases of psittacosis detected by real-time PCR

B.M. de Jongh, J.A. Kaan, A.J. Meinders, J.J. Mager, E.J. van Hannen *(Nieuwegein, NL)*

Objectives: Psittacosis can be a life threatening zoonosis caused by an infection with the obligate intracellular microorganism *Chlamydophila psittaci*. Although the majority of these patients presents with typical symptoms like abrupt onset of fever, rigors, sweats, prominent headache, and couch, about 18 percent do not show any respiratory symptoms. Culture is difficult and time consuming. Isolates of *C. psittaci* are highly infectious and the isolates should be handled under bio-safety level 3 conditions. Molecular techniques like real time PCR are therefore ideal alternatives for the detection of *C. psittaci* to aid the diagnosis of psittacosis.
Methods: We developed an internally controlled real time PCR system that targeted the ribosomal intergenic spacer of *C. psittaci*. The PCR assay was validated on a set of clinical samples from serologically confirmed patients with psittacosis and controls.
Results: Using this PCR system we detected an outbreak of psittacosis among members of a veterinary unit. One of these members was admitted to ICU because of multi organ failure.
Conclusion: With a new developed real-time PCR for *C. psittaci* we were able to identify three patients with psittacosis related to a common source. The symptoms of the patients varied from mild to severe. This real-time PCR for *C psittaci* enables the diagnosis of psittacosis in prior clinical undetected cases.

P1045

Audit of sputum samples before and after a national smoking ban

J. Fennell, B. Cryan, G. Corcoran *(Cork, IE)*

Objectives: Smoking is associated with increased morbidity and mortality from cancer and cardiovascular disease. Smoking is also associated with infections such as TB, exacerbations of COPD and meningococcal disease. In March 2004, Ireland became the 1st nation to enforce a universal smoking ban in the workplace. This provided a unique opportunity to assess whether the change in population smoking practice affected the number of respiratory tract infections and the epidemiology of respiratory pathogens. This would help to show if the smoking ban provided any shorter-term benefits other than the predicted long-term benefits in cancer and cardiovascular disease, and may suggest if a reduction in smoking protects against any specific pathogens.
Methods: Cork University Hospital is a tertiary referral centre serving a population of 500 000. Samples are evenly divided between inpatient and GP samples. Sputum samples for culture and sensitivity from June 2002–March 2005 (22 months before and 12 months after the imposition of the smoking ban) were examined. Information on smoking patterns was provided by the Office of Tobacco Control. Sputum samples, tracheal aspirates and bronchial washings were processed using standard accredited laboratory methods and results logged into an APEX (iSoft) database, queried using Cognos database analysis software and analysed using Excel (Microsoft). Results from children under 18 and further positives from each patient were discarded. The monthly totals and the number of positive samples for *Streptococci pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae* and *Moraxella catarrhalis* were assessed and analysed.
Results: 34342 samples were processed of which 19855 (58%) were positive. The number of positive samples was 5764 (60%) from June 02–March 03, 7020 (56.4%) from April 03–March 04 and 7071 (57.6%) from April 04–March 05. The monthly means of the number of selected species before and after the smoking ban are shown below:

Mean No. of Positive Sputum Samples/Month in the Community:

	H influenzae	*S pneumoniae*	*S aureus*	*M catarrhalis*
Prior to Ban	19.73	9.18	8.41	5.95
After Ban	19.5	6.25	9.08	5.75
Percent change	-1.15	-31.93	+8.02	-3.44

Mean No. of Positive Sputum Samples/Month in the Hospital:

	H influenzae	*S pneumoniae*	*S aureus*	*M catarrhalis*
Prior to Ban	29.05	7.95	34.64	8.86
After Ban	33.17	11.58	35.67	7.00
Percent change	+14.19	+45.62	+2.97	-21.03

Conclusion: The smoking ban was associated with substantial changes in the prevalence of certain bacteria, notably *S. pneumoniae* for which the changes in the community were mirrored by an opposing change in the hospital sputa results. Further work is required to see if these changes were reflected in the mortality and morbidity from respiratory tract infections during this period and to explain the apparent inverse relationship between the changes in community and hospital specimens of *S. pneumoniae*.

P1046

Prognostic factors in patients with pneumococcal bacteraemia in a county teaching hospital, Spain

G. Peralta, E. Lopez, J. Pelayo, M.P. Roiz, I. De Benito, C. Fariñas, M.B. Sanchez, J.C. Garrido *(Torrelavega, ES)*

Objectives: Despite medical advances pneumococcal bacteremia has an important mortality with prognostic factors that have been addressed in several studies. However most of these data are provided by studies developed in University hospitals, with a high incidence of co morbidity, and the prognostic factors were obtained by a univariate analysis.
Methods: All cases of *S. pneumoniae* bacteremia detected from January 1996 to June 2005 were identified using the microbiology laboratory database. A standardized data collection form was used to review the hospital records. In statistical analyses, Student's t test was used for the comparison of mean values and chi square test and Fisher's exact test for the comparison of categorical data (two tailed). A stepwise logistic regression analysis with a progressive conditional incorporation of variables was performed for multivariable analysis.
Results: Of the 115 cases of bacteremia detected in the study period, 14 (12.2%) died. All of the pneumococcal bacteremias were community acquired. In a univariate analysis, factors associated with mortality were: age (63.3 ± 18.5 yr among

survival vs. 76.9 ± 11.2 yr among who died), duration of illness at admission (3.2 ± 3.6 days vs. 2.2 ± 1.4 days), the absence of fever (6.9% vs. 35.7%), the absence of chills (63.4% vs. 92.9%), the presence of severe sepsis or shock (7.9% vs. 28.6%) and the presence of bilateral pneumonia (9.2% vs. 42.9%). In the multivariable analysis factors associated with mortality were: the presence of bilateral pneumonia (OR: 12.7, IC95%: 2.4–66.4, p = 0.003, and the absence of fever (OR: 7.3, IC95%: 1.4–36.9, p = 0.02). The duration of illness at admission (OR: 1.5; IC95%: 0.99–2.3, p = 0.053) reached statistical borderline significance.
Conclusions: Among our patients with pneumococcal bacteremia, mortality is low. The duration of illness at admission, the presence of bilateral pneumonia, and the absence of fever are prognostic factors.

P1047

Ambulatory treatment with telithromycin versus clarithromycin of community-acquired pneumonia in Spain

B. Puchades *(Valencia, ES)*

Objectives: To assess the efficacy and safety of telithromycin versus clarithromycin in the ambulatory treatment of community-acquired pneumonia in patients not requiring hospitalisation.
Patients and Methods: 192 outpatients with Fine I or II community-acquired pneumonia were randomised to receive telithromycin 800 mg od for 7 days (95 patients) or clarithromycin 500 mg bid for 10 days (97 patients). Patients requiring admission to hospital were not included. Visits were performed during and post-treatment. Final evaluation was performed 17–24 days after inclusion in the study. Failure was considered when one of the following conditions was present: persistence or progression of CAP sign/symptoms or X-ray, death, severe adverse events precluding treatment compliance or the need of a different antimicrobial agent.
Results: Patients were in a range of 18–73 years of age, with 101 male and 91 female patients. A significant difference ($p < 0.05$) in success rate of 9.1% (95%CI 0.1, 18.2) between treatments was found in the final clinical assessment (92.6% with telithromycin versus 83.5% with clarithromycin). Failure rates were 6.3% with telithromycin and 12.4% with clarithromycin. Distribution of the 12 failures with clarithromycin was asymmetric, 50% of them in the first 48 h. Of the 6 telithromycin failures, only 2 occurred in the first 48 h. No differences were found with respect to frequency or severity of adverse events between both study drugs.
Conclusions: Telithromycin for 7 days showed higher efficacy than clarithromycin for 10 days in the empirical ambulatory treatment of Fine I and II community-acquired pneumonia in Spain, where there is a need to cover atypical pneumonia in addition to penicillin/macrolide resistant pneumococci. Both drugs were equally well tolerated.

P1048

Comparative assessment of levofloxacin and macrolides in acute exacerbation of chronic bronchitis: clinical efficacy and symptom-free intervals

L. Dvoretsky, N. Dubrovskaya, S. Yakovlev, S.A. Grudinina, O.Y. Filimonova, S. Sidorenko *(Moscow, RU)*

Objectives: Comparative clinical studies usually show similar clinical effectiveness of different antibiotics in acute exacerbation of chronic bronchitis (AECB). At the same time the most important outcome of AECB therapy is the length and full value of the disease remission. In connection with this it seems useful to estimate the influence of antibiotics on the long-term prognosis for patients with AECB, i.e. the length of symptom-free period after disease exacerbation.
Methods: It was the open randomised comparative study. Patients with AECB who had at least three exacerbations per year were included into the study. One group of patients with AECB were treated by levofloxacin (LFX) 500 mg per day during 5 days and the other group-by macrolide antibiotic (azithromycin or clarithromycin) during 5 to 7 days. After the treatment the patients were followed up for 12 months.
Results: A total of 49 patients with AECB were included into the study, 29 of which were treated by LFX, and 20-by macrolide antibiotic. The average age of the patients was correspondingly 58.7 and 57.2 years, length of chronic bronchitis-15.4 and 11.1 years. Number of AECB in previous year was similar in both groups. Clinical cure rate estimated at 30 days after the end of treatment was 96.6% for LFX and 90.0% for macrolides. The eradication rate of causative microorganisms (*Haemophilus influenzae* was the main pathogen) was correspondingly 100 and 30%. The regression rate of main symptoms of exacerbation (cough, dyspnoea, sputum volume) was higher during LFX therapy. During the follow-up period of 12 months the incidence of AECB (requiring prescription of antibiotics) was 53.6% in the group of patients who were prescribed LFX, and 88.9% in the group of patients who were treated by macrolide antibiotics ($P < 0.05$). Average length of symptom-free period after LFX and macrolide treatment was correspondingly 289 ± 90 and 165 ± 112 days ($P < 0.01$).
Conclusion: LFX was superior to macrolides in long-term prognosis in AECB and prolongs the symptom-free period.

P1049

Lower respiratory tract infections caused by *Haemophilus influenzae*

D. Kofteridis, D. Alegakis, G. Chrysofakis, T. Anastasopoulos, A. Repa, S. Maraki , G. Samonis *(Heraklion Crete, GR)*

Purpose: The description of the clinical features of lower respiratory tract infection (LRTI) caused by *Haemophilus influenzae* among hospitalised patients.
Materials and Methods: The medical records of hospitalised adults patients with *H. influenzae* LRTI during a 7 year period (1996 to 2002) were retrospectively reviewed using a standardized questionnaire.
Results: Thirty-four men (76%) and 11 women (24%) were identified suffering of *H. influenzae* LRTI. Their median age was 68 (range 28–86 years). The majority of patients had smoking history (38; 84%) as well as an underlying condition. Chronic obstructive pulmonary disease in 26 (58%) was the most frequent. In 34 patients (76%) the infection manifestated as pneumonia. Among them 9 (26%) had lobar, and 22 (49%) segmental opacification in the chest x-ray. Pleural effusion accompanying parenchymal lesions was observed in 7 patients (15.6%). All patients were treated empirically with antibiotics. Based on the results of the sputum cultures the regimens were proven appropriate in 40 patients (89%). Thirty-six *H. influenzae* isolates (84%) were resistant to amoxycillin, 15 (34%) to co-amoxiclav and 3 (7%) to ciprofloxacin. In forty out of the 45 patients (89%) the infection had a favourable outcome. All five patients who died had pneumonia with respiratory failure and a severe underlying condition.
Conclusions: LRTIs due to *H. influenzae* occurs in populations with underlying disease. A high rate of amoxycillin resistance and an alarming increase of resistance to other antibiotics used were observed.

P1050

Outcomes and diagnostic workups for patients with community-acquired pneumonia according to Pneumonia Severity Index categories: results of a large single-year series

E. Pérez-Trallero, A.M. Martín-Sánchez, R. Dal-Ré, J.E. Martín-Herrero, J. Garau, F. Baquero *on behalf of the NACER Group*

Objectives: To assess variations in outcomes in patients hospitalised with community-acquired pneumonia (CAP) according to Pneumonia Severity Index (PSI) categories.
Methods: Retrospective review of the charts of patients admitted to the hospital with the diagnosis of CAP over a 1-year period (1Nov01 to 31Oct01) in 10 geographically scattered hospitals in Spain. To our knowledge this is the largest single-year series in Spain, and one of the largest in the world (if not the largest) when adjusted by population.
Results: Overall 3233 patients with CAP were initially included in the study. PSI score were properly recorded in only 1722 (53.3%) patients. The proportion of pre-treatment blood cultures was higher among patients in low-risk classes (I–III) than it was for those patients in high-risk classes (IV–V) (52.4% vs. 45.2%; $P < 0.01$), whereas the proportion of blood cultures with a positive result was lower in the former group (9.4% vs. 14.1%; $P = 0.03$). Significant differences between both classes were also observed in the proportion of other diagnostic techniques applied, such as sputum cultures (52.4% vs. 38.7%; $P < 0.01$), *Legionella* urinary antigen assay (49.0% vs. 32.0%; $P < 0.01$) and pneumococcal urinary antigen assay (38.5% vs. 26.3%; $P < 0.01$). Outcomes according to PSI categories are shown in the Table.

Outcome measure	Patients in PSI risk class					
	I	II	III	IV	V	Total
Patients (%)	201	255	357	623	286	1722
Early death[a]	0.0	0.4	0.6	0.6	8.7	32
Total deaths	0.5	2.0	3.1	6.9	26.6	136
Admission ICU	2.0	3.5	5.3	5.6	10.8	98
Mechanical ventilation	0.5	0.8	3.6	4.0	6.3	59
LOS, mean days[b]	8.2	9.2	10.9	12.7	14.4	11.5

NOTE: Data are % of patients, unless otherwise indicated. ICU, intensive care unit.
[a] Died <= 48 h after admission to the hospital.
[b] Calculated from data for survivors; all durations are from the time of hospital admission.

Conclusions: - All diagnostic workups were performed more frequently in low-risk patients than in high-risk patients.- In spite of ATS and IDSA guidelines recommending to carry out a *Legionella* urinary antigen in severe CAP, in our study this test was performed more frequently in low-risk patients (32.0% vs. 49.0%; $P < 0.0001$).- These results suggest that in severe cases antibacterial therapy was prioritised to microbiology and/or that the wider bacterial spectrum covered by empirical therapy provided the physician with a lower need to obtain etiological diagnoses.

P1051

Factors influencing length of hospital stay in a large, single-year series of community-acquired pneumonia

E. Pérez-Trallero, F. Baquero, C. García-Rey, J.E. Martín-Herrero, A.M. Martín-Sánchez, J. Garau on behalf of the NACER Group

Objectives: To identify variables associated with length of hospital stay (LOS) in patients with community-acquired pneumonia (CAP) who were discharged from hospital
Methods: Retrospective review of the charts of patients admitted to the hospital with the diagnosis of CAP over a 1-year period (1Nov01 to 31Oct01) in 10 geographically scattered hospitals in Spain. Data were available from 3233 patients. Patients who died were removed from the analysis. Multivariate Cox regression analysis was performed to assess factors independently associated with more prolonged LOS.
Results: Overall mean LOS was 11.5 days (95%CI: 11.2 to 11.8), with a median 9 days (Q25: 7 days; Q75: 14 days). Range 1 to 111 days. Ninety-six point two patients stayed ≤ 28 days. The site-specific mean LOS ranged from 7.7 to 16.0 days. The Table shows the results obtained for the different variables assessed.

Variable	Hazard risk (95% CI)	P value
Blood cultures result	1.33 (1.08-1.65)	0.009
ICU admittance	2.37 (1.89-2.99)	<0.001
Hypoxia	1.28 (1.17-1.39)	<0.001
X-ray multilobar CAP	1.23 (1.09-1.39)	<0.001
Age (>= 65yr)	1.38 (1.25-1.52)	<0.001
Fever	0.91 (0.84-0.99)	0.041
Comorbidities	1.09 (1.00-1.19)	0.048
Active drinker	1.38 (1.16-1.64)	<0.001
Hospital center	N.A.	<0.001

Conclusions: - Admission to ICU, positive blood cultures, hypoxia, multilobar X-ray involvement, age ≥ 65, absence of fever, comorbidities and alcohol abuse (all of them know criteria of severity) increased significantly LOS.
- Neither empirical antibiotic choice as by IDSA/ATS guidelines, etiological diagnosis, nursing-home stay nor active smoker had an insignificant influence in LOS of those who survived.- Likewise, hospital centre was an independent factor associated to LOS. Variations among centres suggest an increase in medical care costs in hospitals with the largest LOS.
- The identification of factors that increase LOS in patients with CAP and especially those that are not attributable to differences in patient's characteristics, if opportunely modified could mean an important saving

P1052

Physicians' attitude towards guidelines and on choice of regimen for respiratory infections

T.A. Peppas, O. Kosmopoulou, I. Binikos, I. Skoura, I.F. Hager, S.I. Pappas, N. Galanakis *(Piraeus, GR)*

Objective: To assess physicians beliefs on respiratory tract infections guidelines (G) and whether they affect their choice of regimen.
Methods: At four different staff meetings of hospital based physicians (HBP) and two educational meetings of community practicing physicians (CPP) a question regarding their attitude towards G and their choice of regimen on scenario of pneumonia (HBP) and pharyngitis and of non-admission warranted pneumonia (CPP) was collected. Anonymity was achieved by ballot voting (HBP) and interactive reply system (CPP). Regimen choices were checked for concordance with major (IDSA, BTS, Hellenic IDS) G applicable on data collection date.
Results: A total of 57 HBP and 127 CPP replies were collected and analyzed. An overall favourable attitude towards G was stated with HBP declaring always (12%) and frequently (76%) following G. The respective rates for CPP were 15 and 64%. The analysis of selected regimens, however, displayed some discrepancy to the statement as only 49% of HBP choice for pneumonia were concordant with current G. Penicillin for a scenario of streptococcal pharyngitis was chosen by 39% of CPP, while their choice for pneumonia was concordant to G at 59% of replies.

Conclusions: Physicians tend to express an overall favourable attitude towards guidelines, but their choice of regimen reflects either a superficial G knowledge, or limited acceptance according to their own beliefs or preferences.

P1053

Influence of aetiologic diagnosis on mortality in hospitalised patients with community-acquired pneumonia: results of a large single-year series

J. Garau, F. Baquero, E. Pérez-Trallero, J.L. Pérez, A.M. Martín-Sánchez, C. García-Rey on behalf of the NACER Group

Objectives: To identify variables influencing early and late mortality in patients with community-acquired pneumonia (CAP).
Methods: Retrospective review of the charts of patients admitted to the hospital with the diagnosis of CAP over a 1-year period (1 Nov 01 to 31 Oct 01) in 10 geographically scattered hospitals in Spain. Data were available from 3233 patients. Multivariate logistic regression analysis was performed to assess factors independently associated with early (within 2 days) and late (> 2 days) mortality.
Results: Overall 280 (8.7% mortality) subjects admitted to hospital for CAP died. By day 28 there were 269 deceased subjects, what represented 96.1% of the total mortality of the series. The site-specific mortality rate ranged from 1.1 to 19.7%. Results are shown in Table.

Variable	Early mortality		Late mortality	
	Odds ratio	95% CI	Odds ratio	95% CI
Blood cultures result	N.S.	N.S.	6.0*	2.6-13.6
ICU admittance	6.3*	2.8-14.2	8.9*	5.6-14.4
Empirical antibiotic¶	N.S.	N.S.	0.6*	0.4-0.9
Hypoxia	2.3*	1.0-5.0	N.S	N.S.
X-ray multilobar	2.2*	1.0-4.7	1.8*	1.2-2.7
Age (≥ 65yr)	3.1*	1.0-9.6	1.8*	1.1-2.9
Fever	N.S.	N.S.	0.5*	0.3-0.7
Etiologic diagnosis	N.S.	N.S.	0.4*	0.2-0.7
Comorbidities	N.S.	N.S.	2.0*	1.3-3.1
Nursing home residence	6.0*	2.6-13.8	1.9†	1.0-3.5
Active smoker	N.S.	N.S.	0.5*	0.3-0.9
Active drinker	N.S.	N.S.	N.S	N.S.
Hospital center	N.S.	N.S.	N.S	N.S.

¶IDSA/ATS first choice; *P<0.05; †P=0.051; N.S, not significant (P>0.05)

Conclusions: Admission to ICU, multilobar X-ray involvement and age ≥ 65 y were independently associated with both early and late mortality. In contrast, empirical treatment choice as by IDSA/ATS guidelines, fever, etiologic diagnosis and active smoking were associated with lower late mortality whereas a positive blood culture and the presence of comorbidities were associated with increased late mortality. Hypoxia and residence in a nursing home were associated with early mortality. In our series, setting the etiologic diagnosis was an independent protector factor of late mortality, but not of early mortality.

P1054

Echo-guided management of complicated parapneumonic effusion in children

C.Y. Chiu, K.S. Wong, Y.C. Huang, T.Y. Lin *(Taoyuan, TW)*

Objective: The optimal management of parapneumonic effusions (PPE) and empyema in children remains controversial; currently there is insufficient evidence to give clear guidance on therapy. The aims of this study were to delineate the biochemical characteristics and to examine the effect of different therapeutic strategies on ultrasound staging of PPE.
Methods: A retrospective chart reviewed at a tertiary pediatric referred center from July 2000 to July 2004. Patients with pneumococcal pneumonia who had complicated parapneumonic effusion or empyema underwent real-time chest sonography and thoracentesis for biochemical analysis were included. The ultrasonic appearances were classified according to the deposition of fibrin or formation of fibrin septations. The staging of ultrasonic appearances of PPE was used to correlate with the hematologic and biochemical variables of pleural effusion. Clinical outcomes of the respective therapeutic strategies on various stage of PPE were also analysed.
Results: A total of eighty-one patients were enrolled in the present study. Chest ultrasound was performed and results were stratified into anechoic fluid (stage 1, n = 23), with floating fibrin strands (stage 2, n = 30) and with septated fibrin (stage 3, n = 28). The mean days of fever elapsed before detection of these stages appeared to be higher with advanced stages (p = 0.03). Univariate analysis revealed that WBC, platelet count in hemogram and pH, glucose, LDH, protein in pleural effusion were significantly associated with the stage of PPE. Further multivariate analysis revealed that pH (≤ 7.2) and protein (≥ 4.1 mg/dL) in pleural fluid were two independent predicting factors for the progression of PPE. Trends in rate of successful tube drainage decreased as the advancement of stages of PPE, especially in patients who had initial chest tube drainage (p = 0.001). Total duration of fever and hospital stay was significantly shorter for those children who had initial video-assisted thoracic surgery (VATS) compared to those who had initial chest tube drainage (p < 0.001).
Conclusions: Chest sonography can well discriminate the progressive stages of bacterial PPE. In children with a progressive PPE with fibrin formation, early aggressive tube drainage may avoid a subsequent surgical intervention. In children with a fibrin septated PPE, an initial VATS is recommended to shorten the duration of fever and hospital stay.

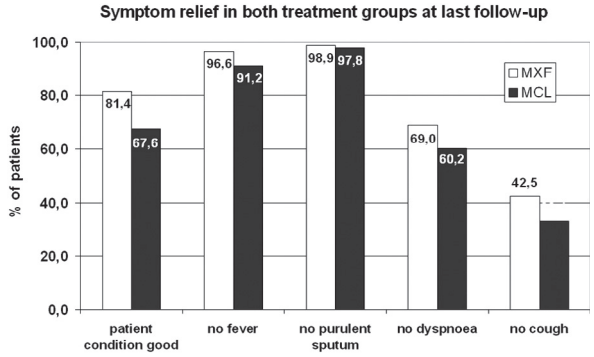
P1055

Real-life treatment of acute exacerbation of chronic bronchitis-moxifloxacin compared to macrolides

T. Schaberg, M. Möller, T. File, K. Stauch, H. Landen *(Rotenburg, Hanau, DE; Akron, US; Leverkusen, DE)*

Objectives: To compare real-life treatment of acute exacerbation of chronic bronchitis (AECB) using moxifloxacin (MXF) tablets or different p.o. macrolides (MCLs) in terms of symptom relief, time until improvement/cure and overall efficacy and tolerability.
Methods: This prospective, non-interventional multicentre study included outpatients with a diagnosis of AECB and a MCL therapy of their last AECB. The current AECB was either treated with MFX tablets or a MCL p.o. The decision about the drug prescribed as well as the dose and duration of therapy was up to the attending physician. Documentation comprised patient characteristics, disease and treatment history, the course of the current AECB treated with MFX or MCL as well as a final assessment of efficacy and tolerability.
Results: In total 1750 patients were included in the analysis, 904 patients were treated with MXF and 846 with a MCL [42.6%

clarithromycin (CCR), 31.2% roxithromycin (ROX), 26.2% azithromycin (AZM)]. Patient characteristics did not differ markedly between the two treatment groups (MXF: mean age of 57.4 years, mean BMI of 26.6 kg/m^2, 27.7% current smokers; MCL: mean age 56.4 years, mean BMI 26.7 kg/m^2, 29.8% current smokers). About 70% of patients had at least one concomitant disease, mostly cardiovascular (MXF group 50.9%, MCL group 45.4%). About 40% of patients in both groups suffered from chronic bronchitis for 1 to 5 years, about 27% for > 5 to 10 years. The mean number of AECBs in the last 12 months was 2.7 and 2.6, respectively. In most MXF patients treatment was applied for 5 (43.8%) or 7 days (42.4%). Main therapy regimens in MCL patients: CCR 500 mg for 4–7 days, ROX 300 mg for 6–7 days, AZM 500 mg for 3 days. Symptom relief in both groups at last follow-up is shown in the figure. Mean duration until overall improvement and cure of AECB was 3.2 days (SD 1.5) and 6.2 days (SD 2.6) in MXF patients compared to 4.4 days (SD 1.8) and 7.5 days (SD 3.0) in MCL patients. 8 MXF patients (0.9%) showed no improvement at all vs. 37 MCL patients (4.4%). 19 MXF patients (2.1%) vs. 86 MCL patients (10.2%) had no recovery. Physicians assessed overall efficacy/tolerability as very good or good in 96.1%/98.1% of MXF patients and 67.5%/91.7% of MCL patients. Both treatment groups showed a similar good safety.



Conclusions: These results of AECB therapy under real-life treatment conditions confirm the superiority of moxifloxacin versus macrolides with a faster symptom relief and higher recovery rates.

P1056

Bulgarian Surveillance Tracking Antimicrobial Resistance – BulSTAR: community-acquired pneumonia: 2004

T. Velinov, M. Petrov, A. Bachvarova, T. Kantardjiev *(Sofia, BG)*

BulSTAR is a National longitudinal surveillance system monitoring the isolation and antimicrobial susceptibility of more important clinically significant microorganisms from blood cultures, cerebrospinal fluid, upper and lower respiratory tract, urine and wound samples in the participating microbiology laboratories. One hundred and three microbiology laboratories – 23 public, 53 hospital and 27 private laboratories from all 28 counties of the Republic of Bulgaria participated in BulSTAR 2004. Data on community-acquired pneumonia are based on 3305 outpatients' lower respiratory tract samples. Participating laboratories used the Clinical and Laboratory Standards Institute (CLSI) methodology. The number of clinically significant isolates is 700. Among them the leading pathogen is *Streptococcus pneumoniae* – 39.1%, followed by *Staphylococcus aureus* – 20.9%, *Klebsiella pneumoniae* – 13.3%, *Moraxella catarrhalis* – 3.9% and *Haemophilus influenzae* – 1.7%. In 2004 the prevalence of penicillin non-susceptible *Streptococcus pneumoniae* was 31.5%, the resistance to erythromycin – 23.8% and to levofloxacin – 0%. The number of methicillin-resistant *Staphylococcus aureus* was 11.6%. Among gram-negative bacteria the sensitivity to most frequently used antibiotics is moderate.

P1057

Clinical manifestations of complicated pneumococcal pneumonia

M.H. Tsai, T.Y. Lin, Y.C. Huang, C.H. Chiu *(Keelung, Taoyuan, TW)*

Streptococcus pneumoniae is the most common cause of bacterial pneumonia in children. During the past several years, the frequency of children with pneumococcal pneumonia complicated by necrosis or empyema is increasing. This study was undertaken to evaluate the clinical characteristics of children with pneumococcal pneumonia, especially those with complicated pneumonitis. Hospitalized children with the diagnosis of pneumococcal pneumonia from January 2002 to October 2004 were enrolled. Patients fulfilled at least one of the evidences of *S.pneumoniae* infection: culture (blood or pleural fluid) yielded *S.pneumoniae* or a positive result of the detection of antigens in the pleural fluid. Complicated pneumonia was defined by the presence of pleural fluid parameters consistent with empyema, and/or CT images compatible with necrotizing pneumonitis. Fifty-two patients were eligible in this study. 53.8% were female and the mean age was 46.0 months. Fever and cough were the most common symptoms. Significantly high CRP (≥100 mg/L) was found in 88.5% of patients. Of the 52 cases, 40 suffered from complicated pneumonia. In the analysis of clinical characteristics, the occurrence of complicated pneumonia was associated with longer duration of fever, length of hospitalization, and longer time to defervescence after antibiotics treatment. No significant difference was noted except CRP in laboratory findings between patients with and without complications. MIC test showed that there was no significant difference regarding the rate of penicillin non-susceptibility between the 2 groups of isolates. Most patients (92.3%) with complicated pneumonia were empirically treated with third-generation cephalosporins or high-dose penicillin and then the regimens were adjusted according to the patients'clinical response. Besides, in cases with complicated pneumonia, managements including decortication. endotracheal tube intubation, and intensive care in PICU were performed in 32.5%,10% and 27.5% of them, respectively. No fatal cases were noted in our series. In conclusion, children with complicated pneumococcal pneumonia may have prolonged course of hospitalization, febrile duration, or longer time to defeverence after antibiotics treatment. Antibiotics-resistant *S.pneumoniae* were not more commonly isolates in patients with complicated diseases. The clinical outcome appears to correlate with the patient's clinical presentation than the susceptibility of the pneumococcus.

P1058

Elderly patients hospitalised with community-acquired pneumonia. Validation and comparative evaluation of a prognostic rule

F. Alcazar, A. Plata, A. Villalobos, I. Perez de Pedro, S. Santamaria, B. Sobrino, L. Valiente, M.A. Garcia-Ordoñez *(Malaga, ES)*

Objectives: To identify prognostic factors of CAP in elderly patients, to generate a discriminating rule to predict hospital

mortality, to validate and evaluate comparative with widely used prognostic index for CAP in general population.
Methods: 809 elderly patients admitted with CAP were selected. Multivariable analyses of derivation cohort (n = 343) identified factors associated with hospital mortality. A predictive model of mortality was established and tested in a validation cohort (n = 476). Our predictive rule was compared with others prognostic indicators for CAP (American Thoracic Society, British Thoracic Society and Pneumonia Patient Outcomes Research Team).
Results: Hospital mortality rates in the derivation and validation cohorts were similar (14.3% vs. 15.5%). The identified prognostic factors were (odds ratio; 95% confidence interval): bilateral radiographic infiltrate (5.2; 2–13.2), a blood urea nitrogen of more than 7 mmol/l (4.3; 1.9–9.4), absence of fever (4.2; 1.9–9.2), a respiratory rate of 30/min or more (4.1; 1.9–9.1), confusion (3.7; 1.6–8.5) and shock (2.8; 1.1–7.2). The discriminating rule to predict hospital mortality comprising three or more of these factors was 94.5% specific, with negative predictive value of 92.2% and had a greater overall accuracy (88.7%) than others general prognostic index (ATS: 44.1%; BTS: 83.2%; PORT: 71.8%; $p < 0.05$) in the validation cohort.
Conclusions: This simple discriminating rule incorporating the prognostic factors identified is powerful predictor of hospital mortality. Widely accepted prognostic indicators for CAP could be not adequate in elderly patients.

P1059

Heart failure and mortality within 90 days after hospitalised pneumonia: a population-based cohort study

R.W. Thomsen, N. Kasatpibal, A. Riis, M. Nørgaard, H.T. Sørensen *(Aalborg, DK; Chiang Mai, TH; Aarhus, DK)*

Objectives: To examine the prognostic impact of heart failure on 90 day mortality in hospitalized pneumonia.
Methods: During 1994–2003 we conducted a population-based cohort study in the three Danish counties of North Jutland, Aarhus and Viborg. All adults hospitalized with a first-time diagnosis of pneumonia were identified in hospital discharge registries and categorized according to whether or not they had a previous discharge diagnosis of heart failure. Through the Danish Civil Registration System we retrieved information about mortality. Information about potential confounders was obtained from the hospital discharge registries and population-based prescription databases. We used Cox regression analysis to compare 90-day mortality rates in patients with and without heart failure, adjusted for calendar year, gender, age, comorbidity, alcohol-related disorders, preadmission antibiotic and immunosuppressive drug use.
Results: The study included 41,793 adults with a first-time hospitalization with pneumonia. Of these, 3824 (9.2%) had a diagnosis of heart failure. The overall 90 day mortality was 21.9%, increasing to 34.2% in patients with heart failure. The adjusted 90-day mortality rate ratio (MRR) from pneumonia in heart failure patients compared with non-heart failure patients was 1.24 (95% CI: 1.17–1.32). The adjusted MMR increased from 1.07 (95% CI: 0.82–1.38) among heart failure patients receiving thiazide-based regimens to 1.49 (95% CI: 1.32–1.68) among heart failure patients receiving loop-diuretic-based regimens including spironolactone treatment. Adjusted MRRs from pneumonia were furthermore higher for heart failure patients with a diagnosis of heart valve disease (1.64, 95% CI: 1.28–2.11) than for heart failure patients with atrial fibrillation (1.34, 95% CI: 1.19–1.51) or previous myocardial infarction (1.06, 95%: CI 0.93–1.22).
Conclusion: Among patients hospitalized with pneumonia, heart failure appears to be associated with increased 90-day mortality. Elevated severity of heart failure may be associated with worse pneumonia outcome.

P1060

Rising incidence and persistently high mortality of hospitalised pneumonia: a 10-year population-based study in Denmark

R.W. Thomsen, A. Riis, M. Nørgaard, J. Jacobsen, S. Christensen, C.J. McDonald, H.T. Sørensen *(Aalborg, Aarhus, DK; Indianapolis, US)*

Objectives: Little is known about temporal trends in the incidence and mortality of pneumonia in the general population. We conducted a population-based cohort study in three Danish counties (population 1.4 million) to examine changes in the incidence and 30- and 90-day mortality associated with hospitalized pneumonia between 1994 and 2004.
Methods: All adults hospitalized with a first-time diagnosis of pneumonia (n = 41,793) were identified in hospital discharge registries and followed for mortality through the Danish Civil Registry System. We determined age-standardized incidence rates and adjusted mortality rates associated with calendar year, gender, age, and comorbidity.
Results: Between 1994 and 2003, the incidence of hospitalized pneumonia among adults increased from 288 per 100,000 person-years to 442 per 100,000 person-years, equivalent to an age-standardized incidence rate ratio of 1.50. The cumulative mortality within 30 and 90 days of admission was 15.2% and 21.9%, respectively, ranging from a 90-day mortality of 2.5% in patients aged 15–39 years to 34.7% in those aged 80 and over. Advanced age was the most important poor prognostic factor, followed by a high comorbidity score and male gender. The adjusted mortality rate ratios among patients with hospitalized pneumonia in 1999–2004, as compared with 1994–1998, were 0.89 (95% CI 0.85–0.94) after 30 days and 0.91 (95% CI 0.88–0.95) after 90 days.
Conclusion: The incidence of hospitalized pneumonia in Denmark has increased considerably during the last 10 years and, combined with persistently high mortality rates, is of clinical and public health concern.

P1061

Once daily sequential intravenous/oral (IV/PO) moxifloxacin is equivalent to IV ceftriaxone plus twice daily IV/PO levofloxacin in the treatment of severe community-acquired pneumonia requiring hospitalisation: the MOTIV study

A. Torres, R. Read, H. Lode, J. Carlet, J.H. Winter, J. Garau, T. Welte, P. Arvis, M.-A. Le Berre, S.H. Choudhri *(Barcelona, ES; Sheffield, UK; Berlin, DE; Paris, FR; Dundee, UK; Hannover, DE; Puteaux, FR; West Haven, US)*

Objectives: To determine the efficacy and safety of once daily 400 mg sequential IV/PO moxifloxacin (MXF) vs high dose ceftriaxone (CTX) plus high dose levofloxacin (LFX) in the treatment of patients with severe community-acquired pneumonia (CAP) requiring hospitalization and parenteral treatment.
Patients and methods: This prospective, randomized, multicentre, multinational, third-party blind, double-dummy trial compared the efficacy and safety of IV/PO MXF 400 mg

OD for 7–14 days, to IV CTX 2 g OD plus IV LFX 500 mg BID, followed by oral LFX 500 mg BID for 7–14 days (LFX dose adjusted for renal function). Patients were stratified to either Pneumonia Severity Index (PSI) Class III (≤ 50% of population) or Classes IV-V. The primary endpoint was clinical cure at test of cure (4–14 days after final dose).

Results: 748 subjects were enrolled, and 738 were randomized (MXF: 372, CTX/LFX: 366). The primary efficacy per-protocol (PP) population was 571 (MXF: 293, CTX/LFX: 278) of which 250 (43.8%) had a microbiologically documented infection at baseline and 337 (59.0%) were PSI Classes IV or V. The demographic characteristics were similar between PP treatment groups. Mean age = MXF: 66.1 16.2, CTX/LFX: 64.9 16.7 years; ventilated subjects = MXF: 4.4%, CTX/LFX: 5.4%. Results are shown in the table. MXF was equivalent to high-dose CTX/LFX even in patients in PSI classes IV-V. In the safety population (MXF: 369, CTX/LFX: 364) treatment emergent adverse events were MXF: 56.4%, CTX/LFX: 53.0%. During IV treatment these were MXF: 40.1% and CTX/LFX: 42.9%. During the study 6.8% of MXF and 4.1% of CTX/LFX subjects died (P = 0.11, NS); no death was considered drug-related by the investigators.

Overall clinical cure rates at the test-of-cure visit (PP population, PSI class IV–V and microbiologically documented population and in subjects with bacteremia) and bacteriological success (microbiologically valid population)

	MXF		CTX/LFX		95% CI
	n/N	%	n/N	%	
PP population	255/293	87.0	250/278	89.9	-8.0%–2.3%
PSI IV–V	145/171	84.8	144/166	86.7	-8.8%–6.0%
Microbiologically-documented population	114/127	89.8	110/123	89.4	–
Bacteremia population	15/20	75.0	18/24	75.0	–
Microbiologically valid population	45/54	83.3	46/54	85.2	–

n, number of subjects with clinical cure or bacteriological success; N, total number of subjects.

Conclusions: Once daily 400 mg sequential IV/PO MXF was shown to be equivalent to high-dose CTX/LFX in the treatment of severe CAP, even in the most severe PSI IV-V group.

P1062

Antibiotic therapy of acute lower respiratory tract infections within Slovakia

K. Banasova, H. Hupkova, V. Foltan *(Bratislava, SK)*

Backgrounds: Acute lower respiratory tract infections (LRTI) are common condition managed in primary care. Although the benefit of an antibiotic therapy is questionable, their prescription is very often.

Objectives: To assess the antibiotic prescription, the assumed aetiology and antibiotic groups prescribed at LRTIs (acute bronchitis, super-infection at acute bronchitis, bronchopneumonia, atypical pneumonia).

Methods: Multicentric prescription study at the acute respiratory tract infections, four weeks in November 2003 in 5 Slovak cities; the same protocols and methodology were applied. The 66 paediatricians registered patient characteristics (gender, age, weight), diagnosis, assumed aetiology, antibiotic therapy, who prescribed antibiotic, risk factors of patients (allergy to penicillin, dysimmunity, chronic respiratory tract disease, etc.).

Results: During four weeks in November 2003, 2056 patients suffered from LRTI. The most common were acute bronchitis (1700 patients; 82.7%), second were super-infection at acute bronchitis (224 patients; 10.9%), third bronchopneumonia (113 patients; 5.5%) and the less frequent pneumonia (19 patients; 0.9%). The incidence of LRTI varied in age groups. The most risk age group were patients in pre-school age (480 patients; 23.3%). Antibiotic treatment was indicated to 1706 patients (83%), the most for pneumonia and super-infection at ac. bronchitis. At the therapy of acute bronchitis and at ac. bronchitis super-infection the co-aminopenicillins were the most indicated antibiotic group (34.9%; 43.7% respectively). Macrolides were used to treat bronchopneumonia and pneumonia (42.7%; 83.3% respectively). GPs conceived mainly bacterial aetiology of infections. The viral aetiology was assumed at acute bronchitis in 9.9% case, at pneumonia 5.3%. At bronchopneumonia, they estimated either viral and bacterial in 40.7% cases.

Conclusions: Although lower respiratory tract infections are caused mainly by viruses, antibiotic therapy is dominant part of treatment (82.7%). Cut-down of antibiotic prescription is possible to accomplish by using in time laboratory examination to settle specific etiological agents of infection.

Sexually transmitted diseases

P1063

Sexually transmitted infections in Kosovo

L. Gashi, N. Ramadani, I. Dedushaj, A. Kalaveshi, I. Humolli *(Pristina, CS)*

Introduction: Control and prevention of reproductive tract infections (RTI) and sexually transmitted infections (STI), are a public health priority all over the world. WHO estimates that 340 million new cases of sexually transmitted infections (STIs) occur worldwide each year. The epidemiological situation concerning STIs, HIV, and AIDS in Kosova is uncertain, characterized by a lack of reliable information on HIV/AIDS/STI and lack of prevention programs and an increased number of possible threats including changes in social and economic situation.

Results: Epidemiological occurrence of RTI/STI in Kosova during the period 1990–2004 did not present the reality of the epidemiological situation because of lack of reporting of RTI/STI cases. It was reported in total 1095 cases. Reporting through this surveillance system identified 21 syndromic diagnoses and 9 cases of *Neisseria gonorrhea* in 2001. As laboratory diagnostics are extremely limited, it is not possible to state the etiologic profile or prevalence of STIs. Since 1986 until December 2002, 41 confirmed AIDS cases with 22 deaths were registered in the Institute of Public Health (IPH) in Pristina, Kosovo. In 2001, 11 AIDS cases were reported; in 2000, six AIDS cases, 4 in 1999 and between 0 and 3 cases during the previous 12 year period. Two-thirds of reported cases were males.

Conclusion: A specific RTI/STI Programme is being established on March 2003. Gaps were identified in various

components of STI prevention and care gnostics are not at uniform current standards. Providers perceive a lack of privacy and confidentiality in STI care themselves. Due to limited supplies and reagents, STI diagnostics is limited. A long-term plan of data needs is essential to direct program work.

P1064

Determinants of human papilloma virus infections in HIV-infected women

S. Ribeiro-Ayeh, D. Labeith, U. Frank *(Mannheim, Freiburg i.Br., DE)*

Objective: To investigate the correlation between cervical dysplasia and socio-demographic and immunologic determinants in HIV positive women.
Methods: Women admitted to a University Hospital as in-patients during the 5-year study period were included in the study. Data were abstracted from clinical charts. Individuals were divided into two groups, those with normal (Pap I-II) and abnormal (Pap III-IV) cervical cytological screening results. Statistical methods were used to compare socio-demographic and clinical variables. Analysis was performed using Statistical Package for Social Sciences (SPSS).
Results: A cohort of 75 women who had tested positive for human immunodeficiency virus (HIV) was included in the study. The mean age was 33.9 (range 19–56). Fifty-one individuals (68.0%) originated from Europe, 16 (21.3%) from Africa and eight (10.7%) from Asia. The overall prevalence of abnormal cytological smears was 29.5%. During the study period, 41 (54.7%) presented with normal and 34 (45.3%) with abnormal smears. Of these 34 women, 27 (79.4%) had cytological evidence of human papilloma virus infection (HPV). Herpes simplex virus infection was detected in seven (9.3%) women, *Trichomonas vaginalis* and *Chlamydia* in five (6.7%) and seven (9.3%), respectively. When comparing both groups, no significant association was found between socio-demographic determinants and the presence of dysplasia. However, a significant determinant for HPV infection was immunosuppression (CD 4 cell count 291 vs 488/μl; viral load 134.679 vs 16.102 RNA copies/μl). Numbers of cytological changes due to HPV infections occurred more frequently in this group (cytological evidence of infection 79.4 vs 24.4%; *Condylomata acuminata* 29.4 vs 7.3%).
Conclusions: Cervical dysplasia due HPV infections appears to be highly correlated with the immunosupression in HIV positive women.

P1065

Infection with HSV in HIV/AIDS patients

L. Manolescu, P. Marinescu, C. Sultana, S. Ruta *(Bucharest, Giurgiu, RO)*

Introduction: Reactivation of latent infection remain one of the main causes of HIV morbidity.
Objective: Prevalence study of herpes virus infection in HIV positive patients monitored in Giurgiu county.
Methods: We evaluated clinical, virusological and immunological 370 adolescents HIV positives with mean age 18 ± 5 years during 1996–2004. The HSV diagnostic was made by immunofluorescence in the Institute of Virology ''St. S. Nicolau''. Each episode of herpes infection was treated with acyclovir, no suppressive therapy was.
Results: The prevalence of herpes virus infection is 56.75%. 98.6% of the patient are HSV-1 infected (facial localisation 87.85% and other localisation 33.2%) and the rest with HSV-2. 50.46% of the patients are treated with different antiretroviral combinations. During monitorisation 67.5% of the treated patients and 61.3% of the untreated presented herpes recurrences (mean 4.2 ± 2.9 vs 3.9 ± 2.7). The recurrences were more frequent in severe immunosuprest patients (CD4<200), 76.4% vs 57.4%, p = 0.005. Recurrent ratio wasn't modified in patients with a good immune response to therapy. The recurrences were less frequent in patients with undetectable viral load, 58.6% vs detectable, 80%, (p = 0.02).
Conclusions: Antiretroviral therapy and immune restoration do not influence the rate of herpetic reactivation.

P1066

Prevalence of HIV, hepatitis B and C and syphilis and risk behaviour among drug users inside and outside prison

C.J.P.A. Hoebe, H.L.G. Ter Waarbeek, C.W. Rademaker, A. Smismans *(Heerlen, Maastricht, NL)*

Objective: The purpose of this study was to evaluate the potential risk for transmission of bloodborne and sexually transmitted infections in Dutch prisons. A high proportion of prisoners in many countries use drugs. Among injecting drug users (IUD) infections like HIV, hepatitis B virus (HBV), hepatitis C virus (HCV) are prevalent.
Methods: Drug users were recruited at different assembly points, such as streetwalkers district, methadon providing points and homeless shelters. Injecting and non-injecting drug users were eligible if they used at least one day a week. Participants were given a face-to-face questionnaire about sociodemographics, prison history, drug using behaviour and sexual practice. Serum samples were tested for the following infection markers: antiHIV, HbsAg, antiHBc, antiHCV and TPPA. HIV antibodies were confirmed by Westernblot. The characteristics of the source population were representive for the regional drug using population: 78% were male, mean age was 36 years (sd. 7; range 19 to 57), 79% was of Dutch origin, 71% had ever used drugs intravenously, 85% used heroin, 59% used cocaine, 65% used methadone, 55% used benzodiazepines, 35% recently injected heroin (in the last 6 months) en 20% recently injected cocain.
Results: We compared two groups: A) 153 participants who were never imprisoned or only once and B.) 165 drug users who were imprisoned at least two times (but often many times more). In group B 10% ever injected drugs while in prison (17/165) and 67% (54/165) recently had sex. Group B differed significantly with higher prevalence of HIV (AntiHIV; 14% vs 4%), hepatitis B (HbsAg; 7% vs 1%; AntiHBc; 64% vs 43%) and hepatitis C (AntiHCV; 72% vs 57%) are found. Syphilis (TPPA) does not significantly differ between the two groups, although prevalences are suggestive in the same direction (3% vs 1%). In our study population 68% (215/318) recently had sex in the last 6 months (usually unprotected) and 37% had sex for money (often without condoms).
Discussion: The high observed in-prison prevalence of viral infections have consequences for in prison transmission potential. Transmission of these infections could be prevented by hepatitis B vaccination among prison populations and prison staff, needle exchange and condom use. Because of the shortage of cells in Dutch prisons, the national policy is changed to put not one prisoner in a cell but two. This policy might create an additional risk for transmission.

P1067

Antimicrobial resistance in *Neisseria gonorrhoeae* in the northeast Romania

A. Badescu, A. Enache, Z. Petrescu, D. Buiuc *(Iasi, RO)*

Objectives: The emergence of gonococcal isolates with reduced susceptibility or resistance to antimicrobial agents is a significant concern in the whole world. This study is aimed to characterize the current antimicrobial susceptibility/resistance patterns of *N. gonorrhoeae* isolates from North-East of Romania.
Methods: From September 2004 to October 2005, a total of 40 isolates of *N. gonorrhoeae* were collected from 37 male patients with urethritis and 3 female patients with cervico-vaginitis attending the Sexually Transmitted Diseases Clinic in Iasi. Specimens from each patient were inoculated directly onto Thayer-Martin selective agar and incubated for 24 to 48 h at 35°C in a 5% CO2 atmosphere. Identification of suspected colonies was based upon the presence of gram-negative, oxidase-positive, superoxol-positive diplococci with typical enzyme patterns. The isolates were also tested for betalactamase production. The antimicrobial agents tested were penicillin, tetracycline, ciprofloxacin, cefixime, ceftriaxone and spectinomycin. Susceptibility testing were performed by agar diffusion method with a GC agar base containing1% Vitox, according to CLSI recommendations.
Results: Of the 40 tested isolates 31(77.5%) were resistant to penicillin by betalactamase production, all the betalactamase negative strains demonstrated intermediate resistance. Tetracycline resistance was observed in 32 (80%) of isolates and 5 (12.5%) were intermediate resistant. 52.5% of the strains were resistant to ciprofloxacin with a further 27.5% showing intermediate resistance. In our study 13 strains (32.5%) demonstrated triple resistance (penicillin, tetracycline, ciprofloxacin). No isolate was found to be resistant to ceftriaxone, cefixime or spectinomycin.
Conclusions: Penicillin, tetracycline, and ciprofloxacin can no longer be recommended for the treatment of gonorrhea in our area; ceftriaxone, cefixime and spectinomycin should be considered the antimicrobial of choice. Since 32.5% of tested gonococcal isolates were resistant to three antibiotics, the monitoring of the resistance to antimicrobial agents in *N. gonorrhoeae* must be established in Romania on a permanent basis in order to adjust standardized treatment regimens.

P1068

Use of polymorphic *Treponema pallidum* repeat genes to elucidate syphilis transmission networks in Amsterdam, Netherlands

S.M. Bruisten, M. Dierdorp, M-E. Kolader, J.S.A. Fennema, R.A. Coutinho *(Amsterdam, NL)*

Introduction: Syphilis incidence has increased dramatically in Amsterdam from 5/100.000 in 1998 to 32/100.000 cases in 2003. This may be due to increased unsafe sexual behaviour of homosexual men resulting from a diminished perceived threat of HIV/AIDS. The TP surface proteins encoded by repeat genes (Tpr) A to L are polymorphic and involved in partial host immunity.
Objective: To develop a typing technique for TP to track syphilis transmission.
Methods: From 2002 to 2004 we studied 199 cases of primary syphilis (96% men). Swabs of genital lesions were used for TP PCR diagnostics. Positive isolates were thereafter used for sequencing analysis of the TprK gene spanning the variable domains V3-V5. Phylogenetic trees of amino acid sequences allowed cluster analysis. Structured questionnaires were used in the Amsterdam STI clinic to collect socio-epidemiological information, and routine contact tracing was performed.
Results: Four patients had two anatomical locations TP-PCR positive on one visit, and 2 patients visited the clinic twice (recidivists) during the study period, resulting in 205 nucleic acid isolates. In 161 (79%) a single TprK sequence type was obtained. In the other isolates either TprK PCR was negative or sequencing implied the presence of at least two TprK types. The isolates of 3 patients with dual positive lesions revealed identical sequences, however in one other patient two different TprK types were found. The isolates of both recidivists also showed two different TprK genes. Despite the fact that none of the TP-patients were known sexual partners, we identified 19 clusters of identical isolates. Cluster size varied from 2 to 4 patients. These clusters may reveal links which remained unknown by classical contact tracing.
Conclusion: Typing the V3 to V5 domains of the TprK gene shows potential to identify transmission routes for syphilis.

Travel medicine, tropical and parasitic diseases

P1069

Animal-associated injuries and related diseases among travellers

P. Gautret, P. Gazin, G. Soula, J. Delmont, P. Brouqui, P. Parola, P. Pandey, D. Kraklau, M. Shaw, G. Brown, J. Torresi, E. Schwartz for the GeoSentinel Surveillance Network

The aim of the study was to retrospectively review the epidemiological and clinical features of mammal-associated injuries in travellers reported to the GeoSentinel Surveillance Network. Characteristics examined included type of animal, countries of exposure, provision of rabies post-exposure prophylaxis (PEP), and the occurrence of other related infections. From May 1997 to May 2005, there were 594 animal-associated injuries reported to GeoSentinel. There was a female predominance amongst injured travellers (M/F ratio 0.84) and the average age was 32 years. The predominant regions from where animal-associated injuries had been reported included Asia (75.1%), Latin America (8.2%) and Africa (6.1%). The animal species associated with injuries were known in 300 cases. Dogs were involved in 61.7% of cases, monkeys in 28%, cats in 8.7%, humans in 1% and bats and mice in 0.6% with no predominance of a particular species in the different geographical regions. Rabies PEP was given in 65% of the cases, however information for the remaining 35% was missing. There were no reports of either rabies or tularaemia in any traveller. Cat scratch disease was reported as a complicating infection in 1.2% of all reports. Animal-associated injuries accounted for 1.4% of the total reported health problems in travellers seen after travel and 1.6% in patients seen during travel. The proportionate morbidity for sustaining an animal bite was higher amongst travellers visiting Asia (2.1%) compared to Africa (0.9%), Latin America (0.6%), Australia-New Zealand (0.9%), North America (1.2%) and West Europe (1.3%) ($p = 0.00001$). Victims of animal injuries were younger than the other ill travellers (32 vs. 35 years old,

$p < 0.0001$) and more frequently females ($p < 0.04$). Finally, the proportionate morbidity for sustaining an animal bite was higher (2.2%) in children less than 15 years ($p = 0.001$). This data shows that animal-associated injuries are not uncommon complaints among travellers presenting to GeoSentinel sites. The highest proportion is recorded in travellers to Asia, mostly in regions which are endemic for rabies, and this led to a requirement for PEP. Pre-exposure rabies prophylaxis must be considered for travel to regions of the world where appropriate PEP is not available. In addition, the pre-travel consultation should also include advice regarding animal-associated injuries, risk of rabies and other associated infection and PEP when travelling to high-risk regions.

P1070

The use of saliva to soothe blood-sucking arthropod bites and the transmission of human herpesvirus 8 (HHV-8)

R. Romano, F. Candura, G. Russo, A. Al Hamdani, L. Ceccherini Nelli, F. Benvegnu', V. Ascoli, M. Coluzzi *(Rome, Pisa, Padua, IT)*

Objectives: The transmission of HHV-8, previously considered a sexually transmitted virus, sometimes appears strictly related to the child's hypersensitivity response to the bite of a blood feeding arthropod and to the mother's (or caregiver's) habits of attempting to relieve itching and reduce scratching, contaminating the bite site with infected saliva (Coluzzi et al. 2002; 2003). Saliva seems to play a major role in the viral transmission and appears to be the reservoir body fluid for HHV-8 (Taylor et al. 2004). In order to confirm the promoter arthropod hypothesis we carried out surveys with questionnaires.
Methods: Two anonymous questionnaires for women in reproductive age and for students were tested on samples from three different regions of Italy (Veneto, Tuscany, Latium), from a region of Palestinian Territories (Gaza strip) and from Africa (Uganda, Cameroon).
Results: The frequencies in the use of saliva among students (age 5–13) were not significantly different in the three Italian regions: Tuscany 13% (20/150), Latium 14.6% (49/335), Veneto 12.5% (47/377). In the case of Tuscany the mothers were also interviewed with closely corresponding results 11.3% (17/150). For the students the mean frequency of 13.4% (116/862) has been compared with the frequency recorded in Gaza strip, 6.6% (6/90) and showed no statistically significant differences ($p = 0.069$). The relatively low rate recorded in Gaza is rather surprising since the socio-economic status of that population is notoriously one of the worst in the Mediterranean area. However it is important to note that among Palestinians there is a culture which tends to ''avoid the use of saliva as it is considered impolite!'' The frequencies recorded in Italy and Palestine were compared with those of Uganda, 74.8% (119/159), showing very high statistically significant differences ($p << 0.00001$). Similar data were also obtained from the comparison with preliminary results in Cameroon.
Conclusion: The results obtained with questionnaires are in full agreement with the known HHV-8 seroprevalences in central Africa (>50%) and in Mediterranean area (10–30%). The present HIV epidemics increases enormously the risk of Kaposi's Sarcoma (KS) in HHV-8 infected people, thus the reduction of HHV-8 prevalence might constitute the primary prevention of thousands of KS cases as one of the most serious complications of AIDS in terms of suffering of the patients and social costs of their assistance.

P1071

Prolonged viraemia and viral shedding of hepatitis E virus in a traveller with fulminant hepatitis

M. Riffelmann, I. Greiffendorf, M. Roggendorf, W. Jilg *(Krefeld, Essen, Regensburg, DE)*

Background: Hepatitis E is generally regarded as a self-limiting and relatively mild disease in non-pregnant travellers. The disease is thought to be at least in part a zoonosis, because one source of transmission may be uncooked meat from wild animals. Fulminant cases have very rarely been reported from non pregnant patients in non-endemic areas such as Japan.
Case report: A 32-year old paediatrician, who travelled with her 33-year old husband and her 36-year old sister for three weeks to Cashmere, India, suffered from diarrhoea in the last week before she returned to Germany. After 4 weeks, she presented with malaise, headaches and fatigue, and after another two weeks she was hospitalized with symptoms of an acute hepatitis. Within a week, she showed all signs of acute liver failure, and a liver transplantation was being prepared. After a total duration of four weeks, however, symptoms and signs of the fulminant acute hepatitis decreased, and after another 4 weeks, the patient was feeling well again, and she was discharged from the hospital. Although her husband and her sister shared all meals with the patients, and travelled together at all times, both stayed without any clinical symptoms, and tested negative for antibodies to HEV and were negative for HEV-RNA by RT-nPCR. Hepatitis E in the patient was diagnosed by detecting antibodies to HEV by enzyme-immunoassay and immunoblot assay. Stool specimens and serum samples were repetitively tested for HEV-RNA by RT-nPCR. It can be assumed that HEV-viremia lasted as long as 4 weeks and HEV-RNA was detectable as long as 9 weeks in stool samples.
Conclusion: This patient was remarkable for developing a fulminant hepatitis E without being pregnant. She shed viral RNA in stools as long as nine weeks after she first presented with hepatitis symptoms. The duration of HEV-viremia detected by HEV-RT-nPCR was estimated be at least 5 weeks after her first clinical symptoms of hepatitis. This case indicates that HEV may be present in patient's blood and stool samples for a prolonged period of time.

P1072

Dengue fever associated with severe reactive thrombocytosis and gangrene of the toes: a case report

S.K. Surrun, D.A. Kandiah *(Singapore, SG)*

Introduction: We present a patient with dengue fever associated with severe reactive thrombocytosis and gangrene of the toes; a condition that to our knowledge has not been described before.
Case report: A 45-year-old Chinese lady, without any previous significant medical history, was admitted with fever, chills, myalgia and skin rashes of a few days' duration. The clinical examination was unremarkable except for some petichaeal rashes on the legs and arms. The blood investigations revealed a mild thrombocytopaenia, the platelet count dropping from 263×10^9 to 135×10^9/l, and a positive paired dengue serology. During the hospital stay she developed severe pain and bluish discoloration of the middle three toes of both feet that progressed to patches of superficial gangrene. The platelet count increased to a maximum of 1299×10^9/l. The

echocardiogram and the CT scan of the aorta were normal. The duplex scan of arteries of the legs was normal up to the distal ends of posterior tibialis and dorsalis pedis. The full auto-immune serology was negative and complement levels were normal. After clopidogrel and pentoxyphylline were started the skin lesions and the platelet count improved significantly.
Discussion: Progression from thrombocytopaenia to severe thrombocytosis in dengue fever is an uncommon event. Though many types of skin lesions are known to occur in dengue fever, gangrene has not been documented so far. In our case, the unusual association of thrombocytosis and gangrene of the toes forced us to exclude other differentials like embolic phenomena, atherosclerosis and immunological disorders. As the investigations were negative, the diagnosis of dengue fever associated with severe reactive thrombocytosis with gangrene of the toes was one of exclusion. Though the thrombocytosis and the clinical condition responded to clopidogrel and pentoxyphylline, spontaneous resolution could still have occurred.
Conclusion: Severe reactive thrombocytosis and gangrene of the toes are rare complications of dengue fever. These complications can occur even in mild cases of dengue fever. A repeat full blood count after an episode of dengue fever is thus of paramount importance.

P1073

Localisation of Rift Valley fever virus in different organs and blood of infected mice using developed PCR test

A. Belov, N. Potapova *(Moscow, RU)*

Objectives: Rift Valley fever (RVF) is an important disease of domestic livestock in Sub-Saharan Africa, causing acute febrile disease, abortions and death. It is also a human disease, usually it shows influenza-like clinical signs. However, during the epidemics RVF may be complicated by hemorrhagic syndrome, encephalitis, blindness and death. RVFV belongs to the genus Phlebovirus, family Bunyaviridae. It has a RNA genome, which consists of three segments. Two segments – large (L) and medium (M) are represented with negative strand RNA, and the small (S) is an ambisense RNA. It is known, that mice are convenient model for studying the virus in a laboratory. The purpose of this study was to detect the RVFV in organs and blood of infected mice using the developed PCR test.
Methods: Vero E6 cells were infected with RVFV (Entebbe strain). Isolation of RNA from monolayer using Trizol® (Life Technologies, USA) and method of RNA isolation based on non-organic carrier (SiO2) was conducted. A PCR in nested modification was developed for this research. Primers were designed based on the sequence of nucleocapsid protein (N) gene following alignment of different RVFV sequences from GenBank database with version 1.6 of Clustal W.
Results: For evaluation of specificity of developed system, different related phleboviruses were tested, as follows: Arbija, Corfou, Karimabad, Sandfly fever Sicilian (SFS), Sandfly fever Naples (SFN), Salehabad and Toscana. Our results indicated that there were no cross-reactions with related phleboviruses. Therefore, the developed PCR test possessed high specificity. For determining of virus localization, different organs (spleen, liver, brain, muscles, lungs) and blood of infected adult mice were tested by using the developed PCR test. Isolation of the virus from the organ tissues of infected mouse was conducted with two different methods. Extraction with non-organic carrier showed the best results, because the virus was detected in all types of tissues. As for the other extraction method, using of Trizol® did not allow to detect the virus in lungs and spleen.
Conclusions: It was confirmed that the virus was present in all tested organs and blood of experimentally infected mice. Also, high specificity of the developed PCR test was shown.

P1074

Presentation of a case with pityriasis rosea and Brucella infection

F. Kaptan, S. Ural, S. El, N. Turker, N.A. Coskun, B. Ormen, G. Yilmaz, M. Ermete *(Izmir, TR)*

Introduction: Although brucellosis is a worldwide existing zoononis, it is more prevalent in the Mediterranean region. Skin lesions in brucellosis are rare and reported in approximately 5% of patients. These lesions are nonspecific and often transient. Pityriasis rosea is a symmetric, erythemateous and squamateous disease, most commonly seen in young adults. Although aetiology is unknown hypersensitivity reaction to human herpes virus 7 is suspected. Histopathologic findings in Pithyriasis rosea are not pathognomonic. The most prominent feature is microscobic vesicle. Oedema in papillar dermis, perivascular infiltration of lymphocytes and histiocytes and extravascular erythrocytes in dermal papilla and epidermis are seen.
Case: Twenty-nine year old, male patient admitted to hospital with the complaints of annular, erythemateous rash on the extremities and both of the buttocks for a month and with fever and knee pain for the last two weeks. During investigations for vasculitis *Brucella* standard tube agglutination test was found to be positive at 1/1280 titre and *Brucella* spp was isolated from the blood cultures. Pityriasis rosea was found in the histopathologic examination of the skin lesions. After starting rifampisin 600 mg/day and doxycycline 200 mg/day, fever returned to normal at the sixth day and the treatment was completed to 45 days in the outpatient basis. Skin lesions resolved after the completion of the treatment. No relaps was seen in 6 months of follow up.
Conclusion: In literature co-existance of brucellosis and Pityriasis rosea has not been reported earlier. Although this co-existance may be coincidental, hypersensitivity reaction to *Brucella* spp. might have been the cause of pityriasis rosea. As a result brucellosis must be one of the diseases in the differential diagnosis of patients presenting with rash and fever, especially in endemic areas.

P1075

Imported chronic Chagas disease in Valencia, Spain: epidemiological and clinical features

P. Segarra, M. García-Rodríguez, V. Abril, C. Parada, E. Ortega, T. Fraile, J. Ballester, M. García-Deltoro *(Valencia, ES)*

Objectives: To analyze epidemiological and clinical features of patients with Chagas Disease in Valencia, Spain.
Methods: Prospective study of patients with Chagas Disease diagnosis between January and October 2005 in Hospital General de Valencia Tropical Medicine Division. Subjects of the study: blood donors or people born in Latin America, children from chagasic mothers, and travellers with epidemiological risk for *Trypanosoma cruzi* infection. Immunological diagnosis was made using commercially available serological tests: Recombinant ELISA (BioElisa-Chagas, Biokit S.A.), that was the test used for serological screening, and IFI (MarDx Diagnostic) used in addition in case of ELISA positivity.
Case definition: Any patient with epidemiological risk factors and two or more different serological test positives. Clinical and

epidemiological review, physical examination, chest radiography, and electrocardiography (ECG) were performed in all cases. Radiographic contrast study of oesophagus and colon and echocardiography only were performed if patient had any symptom or ECG abnormalities.
Results: Ten cases of Chagas Disease have been identified, all of them Latin American immigrants. Seven of them have been submitted from blood bank because of a positive ELISA screening. Mean age 45.5 years (27–66). Gender: 7 females and 3 males. Seven patients had aquired infection in Bolivia, 2 in Argentina, and 1 in Chile. Serological results: see Table. Polymerase chain reaction was available only in 2 cases, and was positive in one. Nine patients were indeterminate chronic forms, and one had a mixed chronic cardiac and digestive form. Chest radiography was normal in all patients. Right bundle branch hemiblock were detected in two patients and left anterior fascicular hemiblock in one case.

Nº	Country	Age	Gender	ELISA	IFI	Symptoms	ECG	XR contrast study	Form
1	Bolivia	66	F	+	1/ 1024	Constipation Palpitations	Ventricular premature beats	Aperistalsis Megaesofagus Dolichocolon	Digestive and Cardiac
2	Bolivia	27	F	+	1/ 1024	Dyspnea	Normal	Normal	Indeterminate
3	Bolivia	29	F	+	1/ 64	No	Normal	N.A.	Indeterminate
4	Bolivia	58	M	+	1/ 512	No	Normal	Normal	Indeterminate
5	Argentina	45	M	+	1 / 64	No	LAFHB	N.A.	Indeterminate
6	Argentina	51	F	+	1/ 64	No	RBBHB	N.A.	Indeterminate
7	Bolivia	44	F	+		No	RBBHB	N.A.	Indeterminate
8	Bolivia	42	M	+	1/ 64	No	Normal	N.A.	Indeterminate
9	Bolivia	43	F	+	>1/ 128	No	Normal	N.A.	Indeterminate
10	Chile	50	F	+	1/ 64	Palpitations Constipation	RBBHB	Normal	Indeterminate

Conclusions: We have identified ten patients with imported Chagas Disease between January and October 2005. Only one has a chronic cardiac and digestive form, and the other nine have indeterminate form. Chagas Disease is an emergent disease in Europe because migratory movements. This situation requires improvement in clinical and diagnostic knowledge, specially in blood banks, and determine priorities on preventive and assistencial needs.

P1076

Anti-*Acanthamoeba* activity of caspofungin

S. Bouyer, C. Imbert, C. Kauffmann-Lacroix, G. Daniault, M-H. Rodier *(Poitiers, FR)*

Objectives: *Acanthamoeba* spp. are causative agents of granulomatous amoebic encephalitis, and more frequently of keratitis, in the case of minor corneal surface injury caused by contact lens or other agents. The effective therapy for these pathologies has to be improved and new drugs are needed, particularly when a resistance to classical therapeutics is encountered. Some azoles or other antifungals have already been used in different clinical situations. We evaluated here the effect of caspofungin, a new antifungal belonging to the echinocandins, on trophozoites and cysts of three strains of *Acanthamoeba*.
Methods: Trophozoites and cysts of *A. castellani* (ATCC 30234), *A. polyphaga* (ATCC 30461), and *A. culbertsoni* (ATCC 30171) were exposed to solutions of 500 μg/ml to 16 μg/ml of caspofungin for 1, 4 and 24 h (trophozoites) and 24, 48 and 72 h (cysts). The viability of trophozoites was then determined by the toluidine blue staining. The cystic forms were resuspended after contact with caspofungin in a medium allowing the viable microorganisms to excyst. Replication of the subsequent trophozoites was observed microscopically during 7 days. Each experiment was repeated three times.
Results: The activity of caspofungin on the trophozoites' growth was dependant of the tested concentration. At the concentration of 16 μg/ml, the viability of trophozoites was reduced of 5 (*A. castellanii*) to 60% (*A. polyphaga*). At the concentration of 500 μg/ml, we observed no viability of the trophozoites for the three tested strains. The cysts of *A. castellanii* and *A. polyphaga* were sensitive to the concentration of 500 μg/ml of caspofungin. As a matter of fact, no trophozoite was recovered after 7 days of incubation. Then, the percentage of viable forms increased when the concentration of caspofungin decreased. Concerning *A. culbertsoni*, the highest concentration used in this study did not prevent the excystation of a weak percentage of the treated cysts forms.
Conclusion: Our results indicated that caspofungin showed *in vitro* activity against trophozoites and cysts stages of the tested *Acanthamoeba* strains. This new antimicrobial agent could be promising to treat, alone or in association, *Acanthamoeba keratitis*.

P1077

Cyclospora cayetenensis infection in five immunocompetent patients in a Turkish university hospital

B. Sancak, Y. Akyon, S. Erguven *(Ankara, TR)*

Objectives: *Cyclospora cayetanensis* (*C. cayetanensis*) is a newly recognized coccidian parasite, emerged as an important cause of epidemic and endemic diarrhoeal diseases in humans. We present, five immunocompetent patients infected with *C. cayetanensis* who sought medical care in Hacettepe University Hospital.
Methods: Three stool samples collected three days consecutively were evaluated in the parasitology laboratory for routine ova and parasite examination. The samples that ova were found as negative, were stained by modified acid fast staining. The ones which revealed pink to reddish stained oval to round organisms 8 to 10 μm in diameter, were diagnosed as *Cyclospora cayetanensis* oocysts. For modified acid fast staining, 2% sulphuric acid was used for decolourisation.
Results: In microscopic examination of the faecal samples, no leucocytes and erythrocytes were seen. Bacteriological stool cultures were found negative. We detected *C. cayetensis* oocysts by modified acid fast staining in five patients. In none of the patients was immunodeficiency determined. All the patients were treated with trimethoprim (160 mg)-sulfamethoxazole (800 mg) bid for 7 days. After treatment, clinical symptoms of patients disappeared between seven to 15 days.
Conclusion: Cyclospora have been isolated in chronic diarrhoea of immunocompetent patients, therefore this infection must be taken into consideration, especially in patients with prolonged diarrhoea. More coccidial infections could be detected if modified acid-fast staining is routinely performed.

P1078

Malaria: current status in Latvia

J. Keiss, I. Zeltina, A. Jeruma, V. Sondore, N. Ivanova, L. Viksna, B. Rozentale *(Riga, LV)*

Malaria is a rare disease in Latvia at present – only 37 malaria patients are registered in 1997 – October 2005. Increasing

tourists, travellers, guest workers flow spread the disease across borders. The aim of study is to characterize the malaria cases, novel malaria tendencies in Latvia.

Patients and methods: Analysis of 34 case records of malaria patients treated in the Infectology Center of Latvia in 1997–2005 was performed. Malaria Plasmodia were diagnosed by using of thin and thick blood smear.

Results: The obtained data show the growing of malaria patients number in Latvia, particularly *Plasmodium falciparum* caused cases. Epidemiological anamneses of 33 out of 34 patients suggested that the patients have been present in malaria endemic region prior to affection (imported malaria). One case of non-imported malaria (local malaria) as a result of *P.falciparum* transmission from imported case has been established. The source of infection could be either a contact person (transmission mechanisms – mosquitoes of the genus Anopheles inhabiting in Latvia; however, sexual contacts and the use of intravenous drugs cannot be excluded) or an infected mosquito that has travelled in from a malaria – endemic area (patient was worker in the railway station). One case of severe *P.falciparum* malaria with broad spectrum of complications (cerebral malaria, acute renal failure, acute respiratory distress syndrome, gastroenteritis, hypoglycemia) and fatal outcome due to late hospitalization (at 7th day of illness) and hyperparasitemia has to be of particular clinical interest. A possible resistance against chloroquine received for malaria prevention has been established in about 60% of the affected.

Conclusions:

1. A continued increasing in malaria throughout the world hastens malaria spread in Latvia: during last years number of malaria, particularly *P. falciparum* caused malaria cases, is rising.
2. The case of local *P. falciparum* malaria is detected in Latvia.
3. The increase in drug-resistant malaria in the world promotes the increase of imported drug resistant malaria in Latvia.
4. Taking into account the widespread distribution of malaria in the world in general and the unconventional transmission routes emerging in Latvia, primary health care physicians should be ready to detect malaria in seemingly non-typical cases.

P1079

Correlation of serum bilirubin level with mortality in *P. falciparum* malaria

A.K. Aribandi, M.S. Albur *(Sheffield, UK)*

According to WHO, severe hepatic dysfunction and encephalopathy are unusual in malaria.However literature review as shown recently some evidence correlating severity of *falciparum* malaria with liver dysfunction.

Objectives: 1) To study liver function abnormalities in patients with *falciparum* malaria who presented with jaundice. 2) To assess the prognosis of *P. falciparum* malaria in relation to liver function abnormalities.

Methods: It is a prospective study done in a tertiary referral hospital (teaching) in south-central India over a period of 2 years. All patients with smear positive *P. falciparum* who presented with jaundice were included in our study. Their clinical presentation along with laboratory findings was noted. Patients with a history of excessive consumption of alcohol, hepato-toxic drugs, known liver disease and/or positive viral hepatitis serology were excluded from our study.

Results: Total number of patients included in this study was 25, with age ranging from 11–55 yrs (mean age 33) out of which 19 (76%) were males and 6 (24%) females. Clinically all patients had jaundice and fever with chills/rigor; 21 patients (84%) had pallor, 20(80%) had hepatomegaly; 13 (52%) had splenomegaly; 10 patients (40%) had hepatosplenomegaly; 16 (64%) had renal failure and 14 (56%) patients had altered sensorium. Based on serum bilirubin level(in mg%, normal range 0.8 to 1.5), patients were categorized into group A (15 patients, serum bilirubin 3–10 mg%), group B (7 patients, serum bilirubin 11–20 mg%), group C (2 patients, serum bilirubin level 21–30 mg%) and group D (1 patient, serum bilirubin 31–40 mg%). Majority (80%) of patients had conjugated hyperbilirubinaemia. Serum ALT levels (in IU/dl, normal range 40 to 80) ranged between 100–324; AST 80–354; Alkaline phosphatase (in KA units, normal range 0–5) 6–24; albumin (in grams/dl, normal range 3.5–4.5) 2–4.5. Overall mortality in our study was 24%. The following table shows correlation of serum bilirubin level with mortality rate.

Serum bilirubin (mg%)	Number	Deaths	Mortality%
2-10	15	2	13.33%
11-20	7	2	28.57%
21-30	2	1	50%
31-40	1	1	100%

Conclusion: Patients with *P. falciparum* malaria & abnormal liver functions, also had other features of severe malaria such as renal failure, ARDS, altered sensorium with high mortality. The mortality rate in this setting was correlating with serum bilirubin level.

P1080

Leishmaniasis in travellers in endemic areas

S. Halichidis, S. Rugina, E. Dumea *(Constanta, RO)*

Objectives: Clinical, epidemiological and therapeutical study about 4 cases of leishmaniasis in travellers in endemic areas.

Methods: Retrospective study about 4 patients with leishmaniasis hospitalized in Clinical Infectious Diseases Hospital of Constana, Romania, during 2005. Diagnosis was histologically and microbiologically confirmed. Treatment consisted of: sodium stibogluconate in one case, meglumine antimonate in 2 cases, and amphotericin B in one case. Clinical follow-up was conducted daily during treatment and 2 months afterwards.

Results: Diagnosis: cutaneous leishmaniasis in 3 patients (2 males and one female) and visceral leismaniasis in one patient (female); age of the patients: between 30 and 55 years old. All travelled in endemic areas. Examination showed nodular lesions and depressed central ulceration all over the body. All lesions and the clinical manifestations (for visceral leishmaniasis) were improved at the end of therapy. For the patient treated with amphotericin B, some brown spots appeared on the hands and soles, on the end of therapy, like adverse event, but disappeared after one week.

Conclusions: Romania is not an endemic area for leishmaniasis, but in the last years cases were related because of the person's circulation in touristic, professional and humanitarian purposes.

P1081

Unusual clinical presentation of visceral leishmaniasis in an immunocompetent patient

T. Kontopoulou, E. Vaidakis, E. Bournakis, N. Alexiou, I. Porfyridis, E. Sioula *(Athens, GR)*

Objectives: Classic visceral leishmaniasis presents as fever, hepatosplenomegaly and pancytopenia. Fever is almost always present at the time of medical consultation. We will present a case of leishmaniasis without fever in an immunocompetent individual.

Case report: A 67-year-old man was admitted in our department due to fatigue, splenomegaly and elevated serum IgG. He never had fever during his illness. In his neighbourhood he had dogs. From his medical history we noted a mild diabetes mellitus, gastritis, appendicectomy, surgical extraction of gall bladder, and thrombocytosis of unknown origin a year before, that yielded without any treatment. Upon admission he had temperature 36.5°C. Physical examination revealed a slight paleness, spleen enlargement (~ 8cm below the left costal margin), small nontender cervical lymphnodes and a maculo-papular rash of the front thoracic side. Blood analysis revealed hematocrit 35%, haemoglobin 12g/dl, leucocytes 3470/mm^3 (granulocutes 40%), platelets 299000/mm^3 (seriously decreased compared to those of a year before), erythrocyte sedimentation rate of 90 mm/h, C-reactive protein 0.4 mg/dl and serum IgG 6870 mg/dl, with a small IgG-k band. Chest radiograph and bone scan were normal. CT scan of chest and abdomen showed only splenomegaly. Bone marrow biopsy and aspirate revealed no haematological malignancy. The bone marrow smear revealed a good deal of *Leishmania amastigotes* and PCR for *Leishmania* from bone marrow was also positive. *Leishmania* serum fluorescent antibodies were positive (1/320). The patient was administered liposomal amphotericin-B 2 mg/kg/day for 5 days and another 2 mg/kg eight days later. After 1 month the patient is doing well, the spleen and lymphnodes are no more palpable, the rash has disappeared and the laboratory values are within normal range.
Conclusion: Mild leishmaniasis –as the one described above- is becoming more and more frequent in the developed and developing world. Therefore, unexplained constitutional symptoms those appear within two years after exposure to an endemic area might warrant investigation for infection with *Leishmania*.

P1082

Efficacy of medical treatment in hydatid disease

I. Dumitru, S. Rugina, E. Dumitru, E. Dumea *(Constanta, RO)*

Objective: To analyze the efficacy of medical treatment for hydated disease.
Materials and methods: We performed a study on 320 patients admitted in our department of parasitology in the last 5 years. The diagnosis was based on ELISA reaction for *Echinococcus granulosus*, chest x-ray, ultrasonography and computed tomography. Treatment involved Albendazole 800 mg/day for 28 days separated by two weeks intervals, minimum three cures. In 2 cases with giant liver cysts (11 cm and 12 cm) we performed a percutaneous ultrasonography-quided puncture of the cyst with fluid aspiration (PAIR) followed by medical treatment.
Results: Echinococcosis had different locations, involving liver (251), lung (81), spleen (6), kidney (5), brain (2), pericardial (2), mediastinal (1), peritoneum (10) and retro peritoneum (3), muscles (4), bone (2), and parotid glands (1). Secondary hydatidosis was reported in 71 cases (22.18%) and multiple cysts were found in 111 cases with liver, pulmonary, muscular, bone, peritoneal and parotid glands hydatidosis and multiple locations in 44 cases. At the beginning of therapy the cysts had less than 7 cm in dimension. After medical treatment we notice a regress of the cysts dimensions or cysts walls calcification in most cases. No significant changes were observed in muscular and bone cysts requiring surgical intervention.
Conclusion: Albendazole is the election therapy in cysts with dimension less than 7 cm. PAIR is a good choice in solitary giant liver cysts.

P1083

The *in vitro* effect of levamisole and praziquantel combination on *Echinococcus granulosus protoscolices*

A. Guvenli, M. Hokelek *(Samsun, TR)*

Objectives: The larval or metasestodes forms of *Echinococcus granulosus* brought into existence a zoonotic infection on humans is called cystic echinococcosis (CE). CE is widely most part of the world and it is also important for human health, animal health and economy. The aim of this study was to investigate the protoscolicidal activities of Levamisole (LVM), Praziquantel (PZ) and Metronidazole (MZ) interactions of E. granulosus protoscolices during in vitro incubation.
Methods: *E. granulosus protoscolices* were aseptically removed from liver hydatid cysts obtained from animals slaughtered at the private abattoir in Samsun, Turkey. Viable *protoscolices* were cultured in medium 199 during three days. The three drugs were added in combination or separately to the medium for comparison of the results. We used the following final concentrations of the drugs: LVM at alone 1000 and 2000 μg/ml, PZ at alone 10 and 100 μg/ml, LVM at 1000 μg/ml + PZ at 10 μg/ml, MZ at alone 100, 50 and 20 μg/ml respectively. Their protoscolicidal activities were assessed after 5, 15, 30, 60 and 120 minutes post-incubation.
Results: A significant protoscolicidal effect was obtained with LVM + PZ combinations after 120 minutes on *protoscolices*. The minimal protoscolicidal effect was found for all MZ concentrations.
Conclusion: Our results showed that LVM + PZ combinations significantly deadly for *protoscolices*, and that further studies with regard to use of LVM + PZ combination in animal models of *E. granulosus* may be of help for designing new treatment regimens for CE in humans.

P1084

Multiple hydatid cysts in the left main pulmonary artery: an extremely rare location

O.K. Aribas, E. Turk-Aribas, F. Kanat, I. Erayman, T. Yuksek *(Konya, TR)*

Hydatid cysts may very seldom develop within pulmonary arteries after ruptured cardiac or hepatic cysts. We report a case with multiple hydatid cysts within pulmonary arteries that necessitated left pneumonectomy. We present a 49-year-old female patient with a symptom of haemoptysis. The patient described fever and cough attacks controlled with medical therapy within previous year. Past history revealed hepatic hydatid cyst operation 5 years ago. Physical examination was unremarkable except diminished pulmonary sounds and inspiratory rates on the left lung. ESR was 68 mm/hour and IHA test for echinococcosis was highly positive. Chest X-ray revealed enlargement of the left hilus, linear heterogeneous infiltration in the left lower zone and a paracardiac pulmonary nodule on the right lower zone. Chest CT scan disclosed $7 \times 5 \times 3$ cm in diameter cystic septated mass lesion obliterating and lying downward within the trace of right main pulmonary artery, widespread tubular dilatation and peribronchial thickening in the left lower lobe bronchi and two small neighbouring nodules in the right lower lobe. A probable crescent moon appearance was seen within the left inferior pulmonary artery. Contrast enhanced chest magnetic resonance imaging revealed multiple cystic lesions in the left main and left lower lobe pulmonary arteries and magnetic resonance angiography showed amputed

left inferior pulmonary artery. Ventilation-perfusion lung scan confirmed perfusion defect in the left lower lobe. Echocardiography was normal. Abdominal ultrasonography revealed heterogenous, hypoechoic calcified area in left liver lobe (operation sequela). The patient underwent left pneumonectomy for irreversible pulmonary arterial wall and pulmonary parenchymal destruction via median sternotomy under total circulatory arrest to prevent contralateral hydatid cyst embolization. Numerous intact or ruptured hydatid cysts in various sizes within the pulmonary artery were observed after pulmonary artery dissection in resected specimen. The intimal destruction was prominent in the dilated and thinned pulmonary artery wall. Albendazole treatment was given to the patient since no procedure was performed on hydatid cysts in the right side. We believe that if an albendazole prophylaxis were given to the patient after surgical intervention to the hepatic cysts 5 years ago such life threatening unusual presentation of hydatid cysts might not be seen and the patient might be saved from pulmonary resection.

P1085

An extremely rare location of cardiac hydatid cyst: interventricular septum

I. Erayman, E. Turk Aribas, S. Avcu, M. Bitirgen *(Konya, TR)*

Hydatid csyt is zoonosis. In humans, disease may usually, manifest by lung, liver involvement and cardiac involvement is rare. Clinical picture depends on localisation of the illness, diameter of the cyst and complications. Echocardiography and radiological imaging are the most reliable methods in describing the anatomical localisation. Serological tests have supportive role in diagnosis. Surgical resection as well as albendazole treatment are suggested in treatment. In this presentation we described a case who did not respond to 6 months of medical therapy. Case who was diagnosed to have cardiac hydatid cyst located at the interventricular septum, was re-evaluated using transoesophageal echocardiography and multislice computed tomography that was recently introduced in our country. 56 years old male patient was applied to out patient clinics of cardiology department with complaints of chest pain, palpitation and abdominal pain. Patient had been taking albendazole 800 mg/day for twenty one days. Transoesophageal echocardiography revealed 4.5 × 3.1 cm hydatid cyst located to interventricular septum. Multislice computed tomography demonstrated 4 × 3 cm hydatid cyst located at interventricular septum. *Echinoccocus* specific IgE was raised 10 times the normal. Six cycles of chemotherapy, each consists of Albendazole 15 mg/kg.day for four weeks followed by 15 day drug free interval, were prescribed.

P1086

A 5-year study of intestinal parasitosis in a general hospital, Piraeus, Greece

S. Konstantopoulou, A. Mourikis, P. Karle, M. Dimitriou, E. Vlachou, F. Vagia, A. Karle *(Athens, GR)*

Objectives: To determine the incidence of intestinal parasites in citizens of Piraeus and analyze the difference between natives and immigrants coming from developing countries. The majority of natives from our hospital area have a low social-economic status.

Methods: During a 5 year period (2001–2005) 5061 faecal samples (4538 from natives and 523 from foreigners) were examined in our hospital laboratory for intestinal parasitosis. Of them 594 were inpatients of the hospital and 4467 outpatients. The 89.12% of the outpatients were workers who were examined in order to obtain a health clearance certificate. All specimens were examined in direct microscopy using wet mount formaline-ether concentration, trichrome stain and P.C.R. methods.

Results: Of 5061 examined specimens, 157 (3.1%) were found positive for pathogen intestinal parasites: *Entamoeba hystolytica*/dispar 136, *Giardia lamblia* 12, *Ancylostoma duodenale* 4 (all patients were foreigners), *Ascaris lumbricoides* 1, *Enterobius vermicularis* 3, and *Taenia sagginata* 1. The incidence of pathogen parasites was 2.64% for greek citizens and 7.07% for the foreign citizens.

Conclusion: The frequency of intestinal parasitosis in Greece is generally low but during the recent years many immigrants have introduced new parasites. It is necessary for the public health services to prevent parasites spreading from the carriers to the healthy population by taking measures of prevention with the right information.

P1087

Filariasis infection in immigrants in a Spanish hospital

N. Portocarrero, A. Amor, M. Baquero, M. Subirats *(Madrid, ES)*

Introduction: In West Africa Filariasis is an endemic chronic disease where is a priority in public health. Some programs for control have been carried out in the last 10–12 years in order to erradicate this disease. The purpose of this study was to investigate the prevalence of imported microfilaries in immigrants coming from the Africa endemic areas in the last 3 years in our area.

Methods: Blood and skin samples from immigrants subjects were collected in our hospital from January 2003 to November 2005. Both Knott concentration technique and observation of skin snips were used for detection of microfilariaes in samples. After them Field's stain was used to confirm diagnosis.

Results: A total of 3763 blood samples and 1259 skin samples (643 from buttocks, 607 from scapulas and 9 from thighs) were analysed. Most of samples are of the subjects coming from Equatorial Guinea. We found 304 positive samples for filarias (6%), 247 in blood (81.25%) and 57 (18.75%) in a skin. The prevalence of species obtained were: we report in 10% of cases with coinfection more than one species of filarias in both blood and skin samples.

Species	Blood	Skin	Total
Manzonella perstans	199 (80.6%)	0 (0%)	199 (65.5%)
Manzonella streptocerca	0 (0%)	4 (7%)	4 (1.3%)
Loa loa	46 (18.6%)	0 (0%)	46 (15.1%)
Onchocerca volvulus	2 (0.8%)	53 (93%)	55 (18.1%)
Total	247 (81.25%)	57 (18.75%)	304 (100%)

Conclusions: Although a recent review of the impact of control programs, indicate that are very effective, we observed a relevant prevalence of filariasis among patients coming from African endemic areas. Routine examination must be performed in people from sub-Saharian Africa.

P1088

Hepatobiliary ascariasis: a case report

A.T. Bastug, A. Erbay, H. Bodur, E. Akinci, M.A. Cevik *(Ankara, TR)*

Objective: Hepatobiliary *Ascariasis* is endemic in third world countries. *Ascariasis* in the hepatobiliary tract can cause acute

cholecystitis, acute cholangitis, obstructive jaundice, acute pancreatitis and hepatic liver abscess. After gaining entry from the ampullary orifice the worms can freely move in and out of the biliary tree and at times the disease can be symptom-less. Ultrasound is an excellent modality to diagnose and follow up hepatobiliary *Ascariasis*. Ultrasound is also useful in confirming exit of the worms from the biliary tree.

Case: A 52 years old male patient was admitted with jaundice. His complaints have begun 15 days ago. In physical examination the skin and scleras were icteric and the abdomen examination was normal. Laboratory examination was revealed an AST: 1414 U/L (normal range 0–40), ALT 1327 U/L (normal range 0–50), Alkaline phosphatase: 1039 U/L (normal range 40–150), total bilirubine: 7.33 mg/dl (normal range 0.2–1.2), direct bilirubine: 5.15 mg/dl (normal range 0–0.4). Viral markers for acute hepatitis were negative. Abdominal ultrasound showed two polyps sized as 4mm on the neck of the gall bladder and wall oedema. At the follow up while the non-infectious reasons of jaundice were researching, the patient vomited *Ascaris lumbricoides* which was measured 18 cm length and then the liver enzymes and jaundice was regressed. The day after vomiting *A.lumbricoides*, the serum transaminases were obtained as ALT: 63 U/L, AST: 28 U/L and total bilirubine was 1.41mg/dl. The control abdominal ultrasound and tomography were completely normal, the polyps which were interpreted as an ascariasis were disappeared. The patient was treated with mebendazole 3×100 mg for 3 days. At the one year follow up the patient had no complaints and ALT and AST levels were normal.

Conclusion: Ascariasis can be a reason of obstructive jaundice and the clinicians should be aware of this possibility especially at the endemic regions.

P1089

The pinworm (*Enterobius* sp.) infection in lung mimicking tuberculoma – case report

H. Järv, V. Adamson, P. Naaber *(Tartu, EE)*

Objective: We report case of human *Enterobius* infection in lung initially misdiagnosed as pulmonary tuberculosis by histological finding.

Case report: A 39-year-old female, working in food market, was routinely checked for tuberculosis by X-ray in March 2004. Chest radiograph showed the presence of a two round opacities in lower lobe of left lung. Latest chest X-ray from year 2001 was without any pathological finding. Patient was seen to pulmonologist for consulting. X-ray finding was confirmed by CT-scan. Diagnostic hypothesis was pulmonary tuberculosis but diagnosis was not confirmed despite to repeated culturing of sputum for mycobacteria from March 2004 to March 2005. In April 2005 patient was hospitalised for diagnostic videothoracosopy. Material from lesions in left lung was sent histopathological investigation in cito. Preliminary finding was showing chronic granulomatous process with necrosis characteristic for tuberculoma. Antituberculotic treatment was initiated. Final histopathological finding demonstrated two granulomas including *Enterobius vermicularis* eggs. Additional analysis from rectal swab and faeces were negative to *Enterobius*.

Discussion: Pinworm (*Enterobius vermicularis*) and tuberculosis are both common diseases in Estonia. Granuloma formation around pinworms and their eggs are quite often reported from appendix, abdominal and pelvic peritoneum, peritoneum of the small and large intestines. Granulomas can form around degenerating adult worms, around discreate eggs, around cluster of eggs and probably around of migrating worms. The granulomas in our case were 1 cm across, having necrosis in centre and other way non-specific, except finding of eggs in centre. Accidental *Enterobius* transmission via oral route into respiratory tract was suspected. We like to emphasize the need to characterize pathogenesis of common parasite like *Enterobius* is, in organs rarely involved. Only few cases of *Enterobius* involvement into lung tissue are characterized in medical literature so far.

P1090

Malnutrition in children in Nairobi's slums

A. Kolenova, A. Ondrusova *(Trnava, SK)*

Even today malnutrition is a major problem in developing countries. Over a third of the world's children suffer some degree of malnutrition. Malnutrition is defined as deficiency of protein, energy or micronutrients, but the commonest nutritional problem is a general deficiency of all food. Malnutrition increases the mortality from common childhood diseases. Malnutrition is not just problem of nutrition, but social and family problem as well. Since January 2003 in Nairobi we recorded 240 children from 0–5 years. After clinical examination, laboratory tests and according strict indication, we placed children in supplementary feeding program. We measured weight, arm circumference at beginning, during (every 2 weeks) and before ending of the program. We checked vaccination schemes, social history. We observed clinical status, risk factors, duration of the program, age of the children, some laboratory results (especially HIV, TB and stool for parasites), and specified the criterions for leaving the program. Mothers were well-informed about conditions of the program, breastfeeding, nutrition, treatment, vaccination; they got breast milk substitutes, multivitamins. In the case of illness, children got a examination and treatment according clinical status. From 240 children, 79% finished the program. Main indication was malnutrition according weight and age, second was contraindication of breastfeeding, because of HIV+ mothers. The most frequent group was children between 1–3 years. From tested children 38% were positive for HIV and 18% for TB. The commonest diseases were respiratory infection, then intestinal infection, skin changes and others. The main intestinal parasite was gardia and a main risk factor was low birth weight. 44% of children leaved the program because of optimal weight and good clinical status, 39% for inadequate co-operation of mothers, 11% died, 6% moved to countryside. Children were often sick and had intestinal parasites. We monitored higher morbidity, complication and mortality especially in children with HIV. After properly monitoring, treatment and education, we achieved a nice success, almost a half of the children leaved with normal weight and good clinical status. But there is still large number of mothers, who don't care and don't understand the problem of properly nutrition. Because of these matters, the most important is to know HIV status (start HAART), to get properly education and to treat all infections.

P1091

Incidence of tropical diseases at university clinic in Mapuordit, Sudan

S. Seckova, A. Augustinova, J. Bencza, M. Bartkoviak, A. Doczeova, M. Taziarova, V. Sladeckova, M. Vilimova, Z. Kniezova, K. Kutna, M. Ocenas *(Trnava, SK)*

Objectives: This observational study presents incidence of infectious diseases and their therapy at DOR (Diocese of

Rumbek) and University of Trnava Clinic in Mapuordit, South Sudan through the patient file analysis in the period from the 1st January 2002 up to 29th June 2003 and monitoring of antimalarial drugs efficiency at the population most vulnerable group in the stable malaria area.
Methods: Case history, clinical examination and laboratory diagnosis determined diagnosis. Data were collected from laboratory registers and hospital clinical records and processed by median test for verification of data level coincidence and test for comparison of two sizeable independent files. In above mentioned period 28 667 patients were examined, from them 22 036 patients (77%) presented infectious disease.
Results: The number of malaria cases or fever of unknown origin was 7445 (33.7%), 2982 of them were in age category from 6 months up to 5 years. Severe malaria with complications was recorded in 185 cases (2.5% from all malarial cases), cerebral malaria was diagnosed in 48 cases, pulmonary oedema in 10 cases, macroscopic haemoglobinuria in 5 cases, generalised convulsions in 67 cases, partial convulsions in 26 cases, severe anaemia in 29 cases. Hyperparasitemia was diagnosed in 230 cases, in the category from 6 months to 5 years in 158 cases. Respiratory tract infections were diagnosed in 3609 patients (16.3%). Sexually transmitted diseases and urinary tract infections were diagnosed in 3339 patients (15.1%), intestinal parasitosis in 3220 patients (14.6%), from which 32% represented ankylostomiasis, diarrhoeal diseases in 1788 patients (8.1%), skin infections in 1886 patients (8.6%), onchocercosis in 589 patients, wuchereriosis in 10 patients, dracunculosis in 8 patients, meningitis in 14 and tetanus in 12 patients. Number of new diagnosed cases of leprosy was 97.
Conclusion: The most common disease remains malaria (caused by Plasmodium falciparum with 76% sensitivity to chloroquine), in this area in early stages easy treatable, but with persistent high mortality rate especially among children under 5 years. The next most common diseases were bacterial infections, especially respiratory tract infections and diarrhoeal diseases. Despite of excellent antibiotic sensitivity and negligible resistance due to low consumption, this group of diseases represents the main cause of morbidity and mortality especially in the most vulnerable group, children under 5 years.

P1092
Saturday night fever
R. Gowda, A. Tunbridge, M. McKendrick *(Sheffield, UK)*

Introduction: Pyrexia of unknown origin (PUO) is one of the most challenging problems for a clinician. If the patient has travelled extensively around the world, the possibilities are legion. Therefore, a careful and thorough history and examination with a methodical, systematic approach to investigation is crucial. We describe an extremely challenging case that highlights the pitfalls in the investigation and diagnosis of PUO.
Case report: A 79-year-old Caucasian man presented with a 2-year history of cyclical fever of up to 39.5c, myalgia, weight loss and night sweats. His fever re-occurred with clockwork like regularity, once a week, every week on Saturdays. During the episodes, the patient felt extremely unwell and was bed bound. However, complete resolution occurred within 24 hours. Inflammatory markers were raised with an ESR of 61mm/hr and C-reactive protein 211 mg/l. A significant travel history around the world working in the navy prompted us to undertake an extensive PUO screen. A wide range of investigations for PUO, imported infections, multiple whole body imaging and bone marrow were undertaken. The only abnormalities were gallstones in the gallbladder and an increased uptake in the right upper abdomen on Indium labelled white cell scanning. It was postulated that this might be the source of fever and the patient underwent a laparoscopic cholecystectomy but this unfortunately did not resolve his symptoms. Despite sequential, empirical therapy with chloroquine, doxycycline and two trials of colchicine (inflammatory familial disorders were considered) his symptoms persisted. He finally had a profound and dramatic response to indomethacin. His symptoms, pyrexia and inflammatory markers all settled rapidly.
Discussion: Our case demonstrates the difficulties of diagnosis and management of PUO. The patient had travelled to numerous exotic locations, which widened the differential diagnosis and prompted us to pursue unusual tropical infections. The results of investigations however, should be carefully evaluated, as subsequent medical/surgical interventions can be potentially dangerous. Our patient had a cholecystectomy without any benefit. Although no cause was found, empirical therapy helped resolve his symptoms and occasionally this may be the only feasible option. We will use this case to discuss the approach to the investigation of PUO.

Seroepidemiology

P1093
Epidemiological features of diphtheria in Belarus
I.A. Karpov, A. Kachanka, M. Kozatchenko *(Minsk, BY)*

Objectives: Diphtheria is an infection, the main clinical manifestations of which are caused by a diphtheria toxin. Diphtheria is referred to as a controlled infectious disease. It is usually detected as a localized form (carrying or the localized forms of larynx diphtheria) and rarely in the form of extensive diphtheria or a toxic form.
Methods: All data refer to the period 1994–2004, and were collected at the Medical State University, Reference Center for Infectious Diseases of the Belarus Ministry of Public Health.
Results and conclusion: According to a thorough bacteriological check-up of the infected and the contacted, the number of carries of toxigenic strains of diphtheria's bacteria out number 100-fold the number of the carriers may amount to 10% and more off those uninfected. Susceptibility to diphtheria depends on the level of antitoxic immunity. At present, as a result of the active immunization of children the disease is characteristic mostly of adults and teenagers who are deprived of their immunity. European WHO committee sets sights on the succeeding objectives – to reduce the number of the diseases to 0.1 per 100 thousand populations by 2010 or earlier. In the period of 1994–1995 Belarus experienced the largest number of the diphtheria acquired – 230 and 322 cases respectively, which accounts for 8.0 and 14.7 cases per 100 thousand populations with 22% of toxic forms. From the year of 1996 till 2001 there was a radical turn in the development of diphtheria epidemic process: the number of the contracted fell from 3.8 to 0.2 cases per 100 thousand populations; in 2000 – 52 cases registered; in 2001 – 25 cases; in 2002 – 7 cases; in 2003 – 6 cases and in 2004 – 15 cases. These data justly the attempts directed to the massive vaccination of those who are subject to it both initially and exercising revaccination. All the individuals who contacted the diseased undergo special chemical prophylactic measures

before the results of the bacteriological tests are ready. The purpose of this is to prevent the disease and the spread of it. The general prescription involves phenoxymethylpenicillin, erythromycin per os according to the age (10 days when we have positive strain) or benzatin-penicillin intramuscularly one time.

P1094

An analysis of the factors effecting the prognose of tetanus in a university hospital between 1991 and 2005

K. Aydin, G. Yilmaz, F. Aksoy, I. Koksal, R. Caylan *(Trabzon, TR)*

Background: Tetanus is a life-threatening infection relatively uncommon in the developed countries but occurs frequently in developing countries with case fatality rates of 40–60%. Early diagnosis and management may be life saving. In this study is made an analysis of the effects of prognosticating factors on the outcome of the patients with tetanus who were seen in our hospital between 1991 and 2005.
Methods: All the patients admitted with clinical diagnosis of tetanus in Karadeniz Technical University Hospital, Trabzon, Turkey, from 1991 through to 2005 were retrospectively reviewed and the data were analysed to determine the socio-demographic features and clinical findings. Analysis was made using SPSS package. Chi-square analysis and student t-test were used for comparison of data.
Results: A total of 44 patients were admitted to our hospital during the study period. All the patients were diagnosed in emergency service by clinical examination. Respiratory support was provided for 24 cases. Only 19(43.2%) patients survived while 25(56.8%) died. The incubation period of the survivors was 12.6 ± 4.8 days while that of those who died was 6.6 ± 4.3 days (P = 0.000). Other factors that significantly influenced survival included severity of spasms (OR = 8.67, 95% CI = 1.83–44.98; P = 0.001), dysphagia (OR = 52.0, 95% CI = 5.04–1301.49; P = 0.000) and provision of respiratory support (OR = 28.0, 95% CI = 4.47–217.6; P = 0.000). When prognostic factors are investigated by logistic regression, the incubation period (OR = 1.41, 95% CI = 1.08–1.85; P = 0.000) and the provision of respiratory support at the time of hospitalization (OR = 0.02, 95% CI = 0.0006–0.39; P = 0.000) were found to be independent prognostic factors for tetanus.
Conclusion: Tetanus is still a severe problem in developing countries. It is a potentially fatal disease. The rate of high fatality in our patients was due to short incubation period and severe clinical findings. The necessity of respiratory support increased mortality to 98%. A one–day extension in incubation period increased the chance of life to 1.41. The majority of tetanus cases occurred among persons inadequately vaccinated or with unknown vaccination history during the acute injury. Primary immunization and scheduled booster immunization are important preventive measures that have greatly reduced the incidence of tetanus.

P1095

Determination of tetanus antibody levels among emergency department patients presenting with injuries

K. Caglar, K. Hizel, A. Altuncekic, A. Demircan, R. Karakus *(Ankara, TR)*

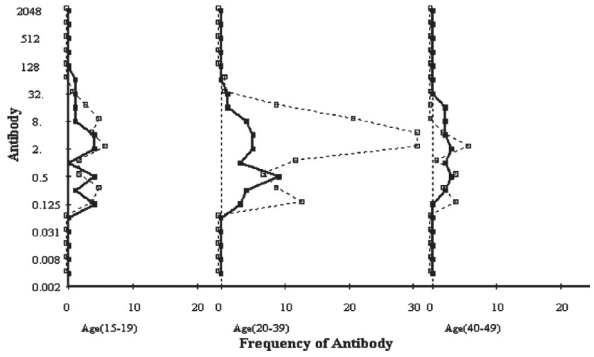
Objective: This study was undertaken to assess the tetanus immunity status in patients presenting to an emergency department of a tertiary care hospital because of accident.
Materials: A total of 155 patients (47 women and 108 men, age from 16 to 81) were enrolled into the study. All patients had various injuries. At each patient's presentation, demographic data and tetanus immunization history given by the patient himself or herself were recorded. Tetanus antibody levels in serum samples of the subjects were measured using a double-antigen enzyme-linked immunosorbent assay. Seroimmunity to tetanus was classified as susceptibility (<0.15 IU/ml), basic protection (0.15–<1 IU/ml), and full protection (≥1 IU/ml).
Results: In terms of protection against tetanus, we found a statistically significant difference between the patients who had a history of completing their primary immunization series and the patients who had not. Among the patients who did not recall their primary tetanus vaccination history, 64.6% of them had antibody titres above 0.15 IU/ml. On the contrary, seroprotection rate (92.2%) was significantly higher (P < 0.001) among the subjects who stated that they had received the primary immunization series. All of the subjects who received a tetanus booster vaccination during last 5 years had protective antibody levels. In overall, 22.6% of the patients were found to be susceptible to tetanus, 23.2% had basic protection, and 54.2% were fully protected. Susceptibility to tetanus among females was found to be significantly (P < 0.05) lower than that among males in emergency department patients. The rate of susceptible subjects among females showed the highest value (90.9%) in those aged +50.
Conclusion: In emergency department patients with injuries, possible risk of tetanus should be taken into account especially in those who did not receive or recall their primary childhood immunization series. In women particularly over 50 years of age with injuries, who are at greatest risk, a booster vaccination should be given in the emergency department practice without any delay in order to avoid leaving many of them unprotected.

P1096

Tetanus immunisation status among women of child-bearing age in three provinces in Turkey

B. Esen, D. Kurtoglu, N. Coplu, A. Gozalan, K. Miyamura, S. Ishida, L. Akin *(Ankara, TR; Tokyo, JP)*

Objective: The goal of the study was to assess tetanus immune status among childbearing aged women in relation to the tetanus vaccine doses in three selected provinces in Turkey.
Method: A total of 2094 subjects were randomly selected from 26 health centres' areas in Diyarbakir, Antalya and Samsun between February 2000 and October 2001. There were no statistically significant differences between provinces by age group, gender and residence (P > 0.05). Of these, 404 15–49 years old women were selected for analyses about tetanus immunity among childbearing aged women. Tetanus antibody titres were determined by using in-house ELISA and particle agglutination tests and antibody titres ≥0.1 IU/ml was accepted as protective level. Statistical analyses were done with qui-square test, Student's t-test, one-way ANOVA, and logistic regression analysis using SPSS 10.0 for windows and the special software prepared using Visual Basic 6.0 and Access.
Results: The women were divided into three age groups, 15–19, 20–39 and 40–49. Among 20–39 years-old women (n = 205), tetanus antibody level was higher in women with 1–3 children than those without children, demonstrating the effectiveness of the pregnant vaccination. The age specific fertility rate in Turkey is typically cumulated among the ages less than 40 years old, and the median age of first marriage in Turkey is 19.5. Based on these data women aged 20–39 years old are accepted as



childbearing aged for analysis. The protective antibody level among 20–39-year-old was 74.7%, and the subjects who received more than two single-type tetanus vaccine doses were 58.8%. Among the provinces studied, Antalya had the highest immunity (P = 0.024). The percentages of the protective antibody level among women 20–39-year-ages were 86.6% in Antalya, 54.8% in Diyarbakir and 78.7% in Samsun. There was an inverse association between the percentage of the immunity level and age (P = 0.002).

Conclusion: Although pregnant vaccination seemed to be effective, the tetanus immunity among women of childbearing age was not enough. There is a need to reinforce the pregnant vaccination through intensive antenatal care services and encouraging pregnant women to receive these services.

P1097

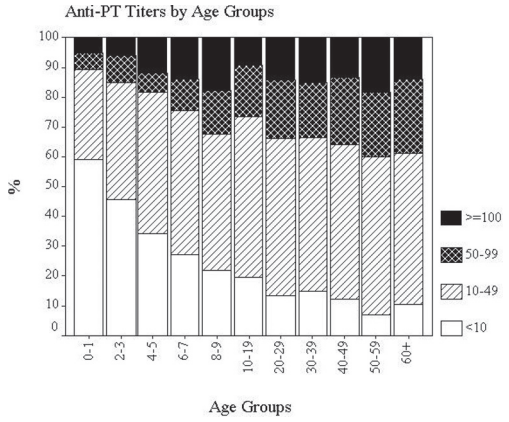
Serological evidence of acute/recent pertussis infection in Turkey: how big is the threat for infants?

B. Esen, N. Coplu, D. Kurtoglu, A. Gozalan, L. Akin *(Ankara, TR)*

Objectives: In Turkey, whole cell pertussis vaccine is administered to 2, 3, 4 months olds and a booster to 16–24 month-old children with a coverage of 83%. Regarding the life-span of antibodies which is 5–12 years and vaccine efficacy that is around 80%, school children, adolescents, and adults become vulnerable to the infection. The aim of this study was to determine the magnitude of acute/recent infection in older individuals and the threat pertussis infection poses for infants. We also wanted to measure the antibody levels in chilbearing aged women to estimate the frequency of passively acquired protective antibodies in newborns.

Methods: Questionnaires and blood specimens were collected from 2085 healthy individuals in three provinces from diverse regions of Turkey. The age groups were between 6 months old and >60 years old. There were no statistically significant differences in numbers of the subjects based on age group, gender, and residence (p > 0.05). The IgG anti-PT antibody was measured by in-house ELISA, 50–99 ELISA Unit (EU)/ml and ≥100 EU/ml were evaluated as acute and/or recent infection, ≥10 EU/ml for protective level and ≥30 EU/ml for chilbearing age women (n = 406) to protect their babies until the first dose of vaccine in 2nd month. Statistical analysis were done by SPSS 10.0 for Windows, Chi-square test and Pearson Correlation.

Results: The anti-PT titres of 50–99 and ≥100EU/ml were lowest in 0–1 year-olds with 5.7% and 5.2%, respectively. This high titres percentage increased by age, and the maximum was 25.0% in ≥60 year-olds for 50–99 EU/ml and 18.5% in 50–59 year-olds for ≥100 EU/ml. In total, there were 15.3% and 12.5% subjects with 50–99 and ≥100 EU/ml, respectively. For unprotected individuals, the situation was the opposite, the highest percentages were observed in 0–1 and 2–3 year-olds group with 59.1% and 45.8%, respectively, and decreased from 4–5 year-olds to ≥60 year-olds between 34,3% and 6.7%. The unprotected subjects were totally 24.6%. Antibody titre ≥30 EU/ml were found in 57.1% of 15–49 year-old women.



Conclusion: There were acute/recent infection in every age-group and lower than 4 year-olds showed high susceptibility indicating risk for serious pertussis infection. Besides, nearly half of the childbearing aged women did not have enough antibodies to protect their newborns. Improvement of primary vaccination procedures and additional vaccination in the older age groups need to be considered for the protection of infants.

P1098

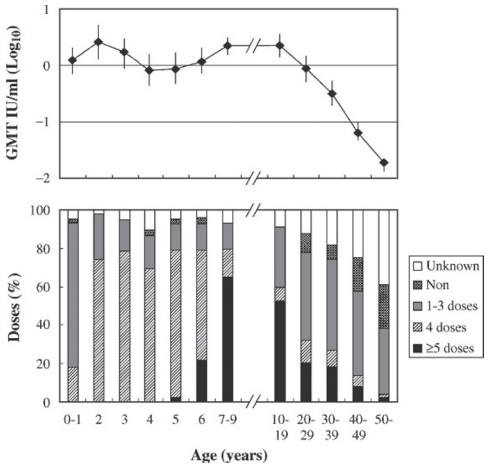
Immunity against tetanus and effect of its vaccination in Turkey

N. Coplu, B. Esen, A. Gozalan, D. Kurtoglu, K. Miyamura, S. Ishida *(Ankara, TR; Tokyo, JP)*

Objectives: The immune status against tetanus in relation to the vaccination was investigated among healthy population in Turkey.

Methods: Questionnaires and blood specimens were collected from 2085 healthy individuals in three provinces from diverse regions of Turkey. The study subjects were between 6 months old and ≥60 years old. There were no statistically significant differences in numbers of the subjects based on age group, gender, and residence (p > 0.05). An in-house ELISA and a particle agglutination test (Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) was performed to detect antibody. From the questionnaires 1789 subjects with vaccination history were evaluated. The statistical analysis was done by using Visual Basic 6.0 and Access, and the Student's *t*-test.

Results: There was a characteristic distribution of the tetanus antibody titres in different age groups. The titres in the groups of up to 20-year-olds were distributed with a peak frequency between 2.0–8.0 IU/ml, but scattered in lower levels in 30-year-olds, and less than 0.01 IU/ml in the groups older than 40s. Geometric Mean Titre (GMT) increased by number of vaccine doses in 0–1 year-olds from 0.205 IU/ml(95%CI;0.066–0.956) for 1 dose to 6.223 IU/ml(95%CI;3.069–12.618) for four doses. Mean vaccine doses for 0–1; 2–6; 7–9 and 10–19 age groups were 2.95; 3.54–3.86; 4.38 and 4.21 respectively, and afterwards the number of doses reduced by age and became 1.21 in ≥50 age group. GMT of the antibody was found 2.54 International Unit (IU)/ml (95%CI;1.63–3.96) for 2 years olds, reduce to be 0.85 IU/ml(95%CI;0.58–1.37) in 4 years olds and became 2.21 IU/ml(%95CI;1.61–2.90) after booster dose in 7–9 years olds (p < 0.01),(p < 0.01). GMT reduced by age and it was 0.019 IU/ml (95%CI; 0.015–0.025) in ≥50 years olds. This



reduction was investigated by the duration after the last dose of vaccination as 0–4, 5–10, 11–19, ≥20 years, and the vaccine doses were evaluated by 1, 2, 3, ≥4 doses. GMT reduced in all of the groups from 1.033 IU/ml (95%CI;0.736–1.442 IU/ml) in 0–4 years to 0.0475 IU/ml (95%CI;0.026–0.086 IU/ml) in ≥20 years group in total. The interception of regression line of X-axis at 0.01 IU/ml (minimum protective level) was 31.6 ± 5.0 years after the last vaccination.

Conclusion: There is good correlation in vaccination and immune status. Besides, although antibodies persist for around 30 years after last vaccination, there is need for additional doses for in adults and the elderly.

P1099

Prevalence of pertussis antibody and vaccination status in three provinces in Turkey

D. Kurtoglu, A. Gozalan, N. Coplu, K. Miyamura, S. Ishida, M. Morita, L. Akin, B. Esen *(Ankara, TR; Tokyo, JP)*

Objectives: The study aimed to asses the immune status against pertussis among healthy population in three geographically different provinces in Turkey in relation to the vaccination status with the whole cell pertussis vaccine combined with diphtheria and tetanus toxoids (DwPT).

Methods: Community-based seroepidemiological survey was implemented in Antalya, Diyarbakir and Samsun between February 2000 and October 2001. Totally 2085 subjects aged 6 months old or above in the 26 health centres were selected using stratified randomised sampling method. There was no statistical difference between three provinces by age group, gender and residence ($P > 0.05$). After obtaining consent to participate, questionnaire and blood samples were collected. Antibodies to pertussis toxin (PT) and filamentous haemagglutinin (FHA) were assayed with in-house enzyme-linked immunosorbent assay (ELISA). Titres ≥10 ELISA Unit/ml are accepted as antibody positive. Statistical analyses were done using SPSS 10.0 for windows and a special software prepared using Visual Basic 6.0 with logistic regression and Chi-square tests.

Results: DwPT vaccination coverage with 3–4 doses among children under 15 year-olds was higher than older ages in every province ($p < 0.05$). This value was significantly lower in Diyarbakir (62.3%) than other two provinces (93.4% for Antalya and 97.7% for Samsun) ($p < 0.05$). However, antibody-positive rates for anti-PT and anti-PT/anti-FHA were higher in Diyarbakir and Samsun than Antalya and for anti-FHA it was higher in Antalya than Samsun ($p < 0.05$). Positive antibody levels for anti-PT and anti-FHA were lowest in the 0–1 year-olds in the three provinces (30.6–46.3%) and increased with age up to 14 year-olds (71.9–94.1%) maintaining among adults. Among ≥ 20 years old anti-PT and anti-FHA positive rates were higher in female than male ($p < 0.05$).

Conclusion: No connection between vaccination coverage and antibody prevalence and the presence of high titred PT antibody among all ages showed wide prevalence of *B. pertussis* infection, suggesting a potential of adults to play a reservoir of *B. pertussis*. It is important to achieve high vaccination coverage especially among infants whose mortality due to pertussis is higher than other age groups. In addition, the immunization of adults such as health workers or family who are likely to have close contact with infants should be considered.

P1100

Analysis of the changes in the tetanus course during the last 40 years – own observations

A. Kalinowska-Nowak, A. Garlicki, M. Bocizga-Jasik, T. Mach *(Cracow, PL)*

Introduction: Tetanus is acute toxicosis, caused by tetanospasmin elaborated by vegetative forms of Clostridium tetani. This powerful toxin inhibits the release of GABA and glycine on alpha motoneuron of spinal cord and brain stem, resulting in increase muscle tone and occurrence of generalised spasm.

Material and methods: Retrospective analysis of 953 cases of tetanus hospitalised in the Department of Gastroenterology and Hepatology and Infectious Diseases from 1965 to 2004. 40 years period of observation was divided at 4 intervals lasting 10 years: I – 1965–1974, II – 1975–1984, III – 1985–1994, IV – 1995–2004.

Results: 953 patients, 442 (46%) women, 511 (54%) men, with tetanus were hospitalised in the Department of Infectious Diseases and Hepatology within 40 years. During the following investigated intervals number of cases of tetanus decreased: I–327, II –276, III–248, and IV–102 patients. Also changes of the patients' age were observed. In the first decade 50 (15%) patients were children less then 15 years old, 260 (80%) were 16 to 70 years old, and only 17 (5%) persons were older then 70 years. 3 (1%) children, 218 (79%) patients aged 16–70 years and 55 (20%) older then 70 years old were hospitalised in the second decade. In the third decade only one child (0.4%) with tetanus were hospitalised. 135 (54.6%) of patients aged 16–70 years, and 112 (45%) were older then 70. During the last ten years, there was no case of tetanus among children, and all patients were older then 30. 41 (40%) among them were younger then 70, and 61 (60%) were older. The mortality rates in the analysed decades were respectively: 28%, 29%, 41%, and 33%. An average mortality rate during the 40 years was 32.5%.

Conclusions: 1. During the last years tetanus is mainly observed among old persons. 2. In spite of modern treatment of tetanus mortality rate is still high, because of advanced patient age and coexisting diseases. 3. Because there is no possibility to eradicate the *Clostridium tetani* from natural reservoir, the proper immunisation is essential, also among old people.

P1101

Seroepidemiology of rubella in reproductive age women in Attica area of Greece during fifteen years (1990 – 2004)

P. Karabogia-Karafillidis, M. Orfanidou, H. Vagiakou, E. Sideris, E. Kontekaki, A. Kefalakis, H. Malamou-Lada *(Athens, GR)*

Introduction: Rubella is usually a mild disease but infection during the first months of pregnancy can have severe consequences as congenital rubella syndrome. The national

rubella immunization programme in Greece was introduced in 1989 and changed after 1999 but no active policy to immunize adults or susceptible women of reproductive age has been implemented to date.
Objectives: The purpose of the study was to determine the current status of rubella immunity in women of childbearing age over a period of 15 years (1990–2004) and to estimate the changes to the immunity status after rubella outbreaks in 1993 and 1999 in Greece.
Methods: We examined a total of 5182 serum samples from women 17–40 years old attending the ''National Reference Center for rubella virus''. The samples were tested for total antibodies by the HAI assay (Rubeo Kit, Biorad) and for IgG and IgM antibodies by an ELISA (Enzygnost, Dade Berhing-dSLabs).
Results: The susceptibility rate for rubella found to be 29.8% (660/2217) for the period 1990–1993, 14.7% (283/1931) for 1994–1999 and 18.5% (191/1094) for the period 2000–2004. In 1993 a large epidemic of rubella and congenital rubella syndrome occurred and was followed by a smaller one in 1999. Acute rubella infection was serologically confirmed in 82 and 53 persons for the years 1993 and 1999 respectively.
Conclusions: The high susceptibility rate for rubella which was observed among women of reproductive age during the period 1990–1993 resulted to the appearance of rubella's outbreak in 1993. Although the sensitivity declined sharply after 1993 a smaller outbreak occurred in 1999. Our results indicate that a comprehensive policy is still needed in order to eliminate rubella and congenital rubella syndrome in Greece.

Mycobacterial infections

P1102

Diagnosis of tuberculosis in suspects in endemic area by using enzyme-linked immunospot assay for gamma-interferon

P.R. Hsueh, J.Y. Wang, C.H. Chou, L.N. Lee, H.L. Hsu, I.S. Jan, P.C. Yang, K.T. Luh *(Taipei, TW)*

Objectives: We evaluate the value of the ex vivo enzyme-linked immunospot assay for gamma interferon (TB-ELISPOT) for rapid diagnosis of active tuberculosis in clinical suspects in endemic area.
Methods: From January to June 2005, patients whose clinical symptoms and radiographic findings were compatible with tuberculosis were recruited and a blood sample was obtained for TB-ELISPOT assay within 7 days after the microbiologic studies were performed.
Results: Sixty-five suspects were studied, including 39 (60%) with active tuberculosis. Thirty-five (53.8%) patients had underlying co-morbid conditions. Thirty-seven patients had positive cultures for M. tuberculosis, whereas nontuberculous mycobacteria were isolated in 11 patients. The overall sensitivity and specificity of the TB-ELISPOT assay were 87.2% and 88.5%, respectively. The positive and negative predictive values were 91.9% and 82.1%, respectively.
Conclusion: Our results showed that the accuracy of the TB-ELISPOT assay for active tuberculosis in clinical suspects is higher than 80%, even in an environment highly contaminated with nontuberculous mycobacteria.

P1103

Autoantibodies to cytokines in patients with mycobacterial infections

G.A. Fernandez-Ramirez, M.E. Rosales, I. Avila-Miyazawa, L. Ceron-Gutierrez, C. Espitia Pinzon, J. Cabiedes, E. Sada, J.A. Montaraz-Crespo, R. Doffinger, G. Barcenas-Morales *(Mexico City, MX; Cambridge, UK)*

Systemic infections due to non-tuberculous mycobacteria (NTM) or BCG have been described in patients with immunodeficiency. In particular, individuals with defects in the IL12 dependant IFNg pathway are selectively highly susceptible to mycobacteria and *Salmonella*. Recently, cases of aquired susceptibity to mycobacterial infection due to high affinity auto-antibodies to IFNg have been described (1).
Objective: Our goal was to further investigate cytokine specific auto-immunity in patients with unusual recurrent and/or disseminated infections by environmental NTM or *Mycobacterium tuberculosis* (Mtb).
Methods: Sera samples included in this study belong to the following groups: NTM or Mtb disseminated infection (n = 62), pulmonary TB (25), Systemic Lupus Erythematosus (SLE)/pulmonary TB (54), SLE (10), vasculitis/pulmonary TB (37), vasculitis (10), salmonelosis (5), recurrent infections due to other opportunistic microorganisms. (15), non-related autoimmune diseases (21), patients on anti-TNFa treatment (4), healthy individuals (60). Cytokine specific autoantibodies to IFNg, TNFa, GM-CSF, IL-1b, IL-6, IL-10, IL-12p40 and IL-18 were screened by Multiple Antigen Fix Immunoassay (MAFI). Samples from patients on anti TNF treatment (Infliximab) were included to control our system. Autoantibodies titer in positive samples were confirmed by direct ELISA.
Results: We detected auto-antibodies to IL-6 (3 cases), IL-10 (8 cases), IL-12p40 (2 cases), IFNg (5 cases), TNFa (2 cases), and GM-CSF (2 cases). No auto-antibodies to any of these cytokines could be found in healthy controls.
Conclusion: Auto-antibodies to cytokines may be present in Patients with unusual recurrent and/or disseminated infections by environmental NTM or Mtb. A potential disease modulating role of these auto-antibodies is discussed.
Reference:
(1) Doffinger, R., Patel, S., Kumararatne, D.S. Human immunodeficiencies that predispose to intracellular bacterial infections. Curr. Opin. Rheumatol. 2005; 17: 440–6.

P1104

Tuberculin skin testing among health care workers in a country with a high tuberculosis prevalence: booster and conversion rates

K. Hizel, F. Ozer, I. Maral, F. Aktas *(Ankara, TR)*

Objective: To determine tuberculin skin test (TST) survey and conversion rates among health care workers (HCWs) of a teaching hospital in Turkey. Thus, to assess whether HCWs are at increased risk for tuberculosis (TB) in a country with a high prevalence and universal BCG vaccination. The effect of previous BCG vaccination on the result of TST was also investigated.
Method: In 2001, two-step TST was administered to consenting HCWs of the hospital. All participants completed a standard questionnaire regarding demographic data including age, gender, workplace, occupation, previous BCG vaccination and history of exposure to tuberculosis. In 2003, TST was reapplied to HCWs with negative reaction after the two-step test.

Results: Of the 513 participants, 364 (70.9%) had a positive TST reaction on initial testing. A higher prevalence of positive TST reaction was found among participants working in the surgical ward (79.9%; OR 2.58; 95% CI 1.50–4.44) than in the medical ward (69.1%), and nurses (75.5%; OR 2.15; 95% CI 1.14–4.06) than clerks (71%), receptionist (68.8%), and administrative personnel (58.9%). Presence of BCG scar was not significantly associated with positive TST response. Among 114 employees with a negative TST reaction who underwent a second test, the booster reaction was found in 12 (10.5%). In 2003, conversion was found in 10 (16.3%) of 61 participants. Conversion was not significantly associated with any of the variables examined.
Conclusion: In high TB prevalence countries, HCWs are increased risk for the infection. The effect of previous BCG vaccination on the result of TST was minimal, and positive response to TST was related to natural infection rather than previous vaccination. Our study supports the CDC recommendations, which emphasize that screening of HCWs with TST is beneficial to evaluate TB exposure and is suitable for high TB prevalence countries too.

P1105

Clinical evaluation of a real-time PCR kit for the detection and partial differentiation of the M. tuberculosis complex

S. Cramer, M. Hess, F.C. Bange *(Hamburg, Hannover, DE)*

Objective: Tuberculosis can be caused by all members of the *Mycobacterium tuberculosis* complex. For a successful treatment the identification of the mycobacterial species being responsible for an infection is important. Classically this is achieved by examining culture material with biochemical and molecular biological methods. We developed a real-time PCR assay (artus^{TM} *M. tuberculosis* diff. LC PCR Kit) for the use with the LightCycler® Instrument (Roche Diagnostics) which allows the detection and partial differentiation of the M. tuberculosis complex directly from clinical samples. To evaluate the assay in total 300 patient samples were retrospectively analysed and the results were compared with standard methods used in tuberculosis diagnostics.
Methods: The real-time PCR assay amplifies a 131 bp region of the mycobacterial nitrate reductase promoter. The amplicon is detected by fluorogenic probes, which allow to differentiate *M. tuberculosis* from other members of the *M. tuberculosis* complex by melting curve analysis. In addition, the assay contains an ''Internal Control'' for the control of the DNA purification efficiency as well as of possible PCR inhibitors. For clinical evaluation 300 samples of respiratory and non-respiratory origin were used, which had been previously analysed by culture and acid fast smear (AFS). 200 of the sample were culture negative for the *M. tuberculosis* complex, 30 were AFS negative, but culture positive and 70 were culture positive and AFS positive. Isolates of mycobacteria were identified by sequencing. From all 300 samples the DNA was purified using the QIAamp® DNA Mini Kit (QIAGEN) and analysed for the presence and differentiation of members of the *M. tuberculosis* complex using the artus^{TM} *M. tuberculosis* diff. LC PCR Kit.
Results: The analytical sensitivity of the assay for the detection of *M. tuberculosis* and the other members of the *M. tuberculosis* complex is 6.4 and 10.4 genome copies per PCR, respectively. The examination of the 300 clinical samples displayed a high sensitivity and specificity for the detection of the *M. tuberculosis* complex and the discrimination of *M. tuberculosis* from the other members of the *M. tuberculosis* complex.
Conclusion: The artus^{TM} *M. tuberculosis* diff. LC PCR Kit is a sensitive and specific tool for the detection of the *M. tuberculosis* complex and the first assay, that allows the highly specific identification of *M. tuberculosis* directly from clinical samples.

P1106

Serodiagnostic potential of mammalian cell entry proteins encoded by mce3 operon of *Mycobacterium tuberculosis*

S. El-Shazly, A.S. Mustafa, S. Ahmad, R. Al-Attiyah *(Kuwait, KW)*

Objectives: The mammalian cell entry (mce) proteins Mce3A, Mce3D and Mce3E encoded by mce3 operon of *Mycobacterium tuberculosis* have previously been shown to be expressed during natural infection in humans and to elicit antibody production in TB patients as determined by Western blotting. This study was carried out to determine the serodiagnostic potential of these proteins by quantitative detection of anti-Mce3A, -Mce3D and -Mce3E antibodies in serum samples from TB patients.
Methods: The mce3A-F genes of *M. tuberculosis* were cloned, expressed as fusion proteins and the recombinant proteins free of the fusion partner were purified. The quantitative detection of anti-Mce3A, -Mce3D and -Mce3E antibodies in serum samples from TB patients (n = 58) was performed by ELISA. The serum samples obtained from healthy BCG vaccinated human subjects (n = 24) were used as controls and the mean absorbance values plus 3 standard deviation for control serum samples were used as cut-off for a positive reaction for serum samples from active TB patients. The serum samples obtained from healthy long term contacts of TB patients (n = 24) were also tested.
Results: Nearly all serum samples (>90%) from active TB patients reacted with Mce3A or Mce3E, 57% reacted with Mce3D alone while 55% reacted with all the three proteins. None of the control sera reacted with any Mce3 protein. The combination of Mce3A and Mce3E detected antibodies in nearly 90% serum samples from active TB patients compared to control human subjects. Although 75%, 42% and 88% of serum samples from long term healthy contacts of TB patients also reacted with Mce3A, Mce3D and Mce3E proteins, respectively, the antibody levels in this group of human subjects were significantly lower ($p < 0.015$) than their levels in TB patients.
Conclusions: The data presented here show that Mce3A and Mce3E encoded by mce3 operon of *M. tuberculosis* elicit strong B cell responses in majority of active TB patients and moderate response in long term contacts of TB patients, the latter grouping likely to be latently infected with this pathogen. The significantly higher antibody levels in TB patients than those found in latently infected individuals could be useful for serodiagnosis of active TB disease while lower levels of antibodies to these proteins in serum samples may be useful for identifying latently infected individuals. Supported by Research Administration grants MI 05/00, MI 02/02 and College of Graduate Studies, Kuwait University.

P1107

Rapid identification of mycobacterial species with test INNO-LiPA mycobacteria v2 isolated from sputum samples obtained from HIV-negative patients

J. Paluch-Oles, M. Koziol-Montewka, M. Dzbrowska, M. Montewka, A. Bogut *(Lublin, PL)*

The nontuberculous mycobacteria (NTM) are a group of bacteria that can cause pulmonary and systemic diseases. An increased

risk of NTB infections is more frequent in patients with acquired immunodeficiency syndrome (AIDS) and HIV–negative patients suffering from immunosuppression due to previous pulmonary disease, old age and alcoholism. For the rapid detection and identification of mycobacteria new strategies have been developed. These novel methods such as HPLC, the BACTEC NAP test and commercial DNA probes are recommended for identification of NTM species. Recently, a new molecular test based on the PCR technique and reverse hybridization procedure has been introduced for simultaneous identification of different mycobacterial species.

Objectives: The aims of the study were to perform the DNA line probe assay – INNO-LiPA Mycobacteria v2 for identification of mycobacterial species obtained from sputum samples collected form HIV-negative patients and to evaluate the risk factors of pulmonary NTM infections.

Methods: We tested 15 NTM strains from patients with pulmonary tuberculosis symptoms, isolated in our centre within the last 2 years. All the strains were processed from subculture on LJ medium, confirmed as NTM using the niacin test and morphology assessment of the organisms. The LiPA assay based on the PCR and reverse hybridization procedure amplificated the 16S–23S ribosomal rRNA spacer region. Subsequently, the biotinylated PCR products were hybridized with specific 22 parallel DNA probes and 2 control lines. The clear pattern of lines allows simultaneous identification of 16 different mycobacterial species.

Results: Among 15 NTM isolates the LiPA revealed 6 isolates identified as *M. kansasii* group I, 4 isolates as *M. xenopi*, and 1 showed a MAIS pattern which also produced a positive result for specific *M. intracellulare* probe. These 3 species were identified in 11 alcoholic patients. In 4 patients older than 60 years 2 strains as *Mycobacterium* spp. and 2 as *M. avium-M. intracellulare-M. scrofulaceum* (MAIS) were identified.

Conclusion: This study demonstrated usefulness of the INNO-LiPA Mycobacteria v2 test for the rapid identification of mycobacterial species and revealed *M. kansasii*, *M. xenopi* and *M. avium*-complex species considered the most frequent mycobacteria causing lung disease in immunocompromised alcoholic and old patients.

P1108

A new automated method for sample preparation of *Mycobacterium tuberculosis* respiratory specimens based on magnetic particles

J. Lysèn, T. Engen, H.K. Høidal Berthelsen, M. Espelund, S. Jeansson, U.H. Refseth *(Oslo, NO)*

Objectives: *Mycobacterium tuberculosis* is today the single greatest cause of mortality due to an infectious agent. Routinely, mycobacterium infections are diagnosed by microscopic examination for the presence of acid-fast bacilli (AFB) and by conventional culture techniques. Detection of mycobacterium by culture takes between from 1 week to 2 months, and AFB testing both lacks sensitivity and specificity. Compared to culture, sensitivity using various available NAAT systems is generally lower. In addition, NAAT based systems require much hands-on time to obtain DNA/RNA of sufficient quality. Thus, the requirement for both a rapid and sensitive diagnostic system for TB diagnostics is still largely unmet.

Method: A pilot study has been performed applying an automated sample preparation for mycobacterium DNA from respiratory samples utilizing magnetic particles. In this system (BUGS'n BEADS^{TM}, Genpoint, Norway), heat-inactivated mycobacterium cells are initially adsorbed to paramagnetic particles and magnetically separated from the sample material. A rapid lysis at RT releases DNA, which is then adsorbed onto the same magnetic particles. After washing, purified DNA is transferred to micro-wells for real-time PCR analysis. As a reference all samples were cultivated in conventional liquid and solid medium up to 8 weeks. A few samples were analysed using the BUGS'n BEADS followed by Roche Cobas Amplicor detection system for *M. tuberculosis* as well.

Results: For smear positive respiratory samples there was full agreement between the BUGS'n BEADS^{TM} isolation procedure combined with PCR detection and conventional cultivation. There was also100% agreement with results obtained using the BUGS'n BEADS^{TM} combined with Cobas Amplicor detection and cultivation.

Conclusion: The results indicate that with the BUGS'n BEADS sample preparation system, sensitivity comparable with cultivation is obtainable for smear positive samples. The results from the BUGS'n BEADS^{TM} procedure combined with RT-PCR or Cobas Amplicor could be obtained within a working day, whereas several weeks were necessary to identify *M. tuberculosis* positive samples by cultivation. Furthermore, the fact that both PCR and the Cobas Amplicor procedure can be used as downstream detection systems, demonstrates that different NAAT may be used in combination with BUGS'n BEADS^{TM}. To further challenge the system, smear negative samples and non-respiratory samples will be included in a more extensive study.

P1109

Gold nanoparticle probe-based diagnostic system for rapid and sensitive detection of *Mycobacterium tuberculosis*

M. Koziol-Montewka, J. Paluch-Oles, P. Baptista *(Lublin, PL; Lisbon, PT)*

Tuberculosis has re-emerged as a major infectious disease in several areas in the world and *M. tuberculosis* (MTb) infections continue to increase worldwide with an estimated annual growing rate of 3%. The application of new methods based on nanotechnology can simplify the bacterial DNA detection and improve MTb identification making the diagnosis of infections caused by this pathogen faster and less expensive than conventional methods. Here we describe a rapid and sensitive colorimetric method based on the use of gold nanoparticles functionalised with thiolated oligonucleotides for MTb detection directly from clinical samples without the prior DNA amplification.

Objectives: The aim of our work was to screen clinical specimens for the presence of MTb using approved INNO-LiPA RifTB kit and a novel gold nanoprobe-based method.

Methods: The used gold nanoprobe-based method consisted on visual and spectrophotometric comparison of the blank (containing the gold nanoprobes alone), negative (containing DNA from samples previously tested and confirmed as negative for MTb) and positive (containing DNA from samples positive for MTb) solutions. After the NaCl addition blank and negative samples turned from the initial pink to a blue–purple colour, denoting gold nanoprobes aggregation. In case of positive samples, the solutions maintained their original pink colour after the NaCl addition. The presence/absence of gold nanoprobe aggregation was corroborated by UV/visible spectra.

Results: We tested 47 clinical samples from patients with the suspicion of pulmonary and extra pulmonary tuberculosis. The gold nanoprobe method proved to give consistent positive or negative results with validated INNO-LiPA RifTB kit for the

ISSN: 1470-9465

MTb DNA detection in 42 out of 47 samples. Five out of 47 samples were interpreted as "non-consistent" possibly due to the DNA content being under the threshold of the method.
Conclusion: The results indicate that DNA-derivatives gold nanoparticles hybridization is a sensitive and specific tool for the identification of MTb directly in clinical samples without prior amplification by PCR.

P1110

Development of tools for detection of DNA and mRNA from mycobacteria in clinical samples

N. Potapova *(Moscow, RU)*

Objectives: Tuberculosis is one of the leading causes of mortality among infectious diseases. The conventional methods for detection of Mycobacteria in clinical samples are based on the demonstration of the acid-fast organisms following cultivation. In the last years polymerase chain reaction (PCR) has been used for detection of *M.tuberculosis* in different tests and has been shown to be at least as sensitive as the classical methods.In this study development of Real-Time PCR-based tests for identification of DNA and mRNA from *M.tuberculosis* is described. Prokaryotic mRNA has a short half-life and can be found only in viable organisms.
Methods: Clinical samples. A total of 70 samples of sputum were obtained from patients treated for a period of severel months. DNA and RNA isolation was conducted with Trizol reagent (Life Technologies) according to the manufacturer's specification. Primers. The specific mRNA target is transcribed from Mtp-40 gene. One of the primers (37 nucleotides) was used for reverse transcription. First 20 nucleotides of this primer were complementary to mRNA. Other 17 nucleotides were chosen randomly and were identical to one of primers for PCR. Two other primers (20 and 17 nucleotides) were used for Real-Time PCR.Reverse Transcription was performed at 42°C. Annealing temperature for PCR amplification of cDNA was 70°C. Annealing temperature for PCR amplification of DNA was 60°C.
Results: Different methods for detection of *M.tuberculosis* in sputum of 70 patients were compared. These were: the developed Real-Time PCR with previous reverse transcription and classical methods (bacteriology and microscopy). The research showed high correlation between the data sets obtained by the PCR and RT-PCR and classical methods.
Conclusions: The developed tools for identification of DNA and mRNA from *M.tuberculosis* could be applied for differentiation of viable and nonviable *M.tuberculosis*. It is useful for rapid diagnostics and monitoring of the efficacy of treatment of tuberculosis.

P1111

Diagnosis of tuberculous pleuritis by nested polymerase chain reaction: a comparison of four different volumes of pleural fluid

N. Jaimchariyatam, K. Kawkitinarong, K. Naweewitphadung, N. Udomsantisuk, S. Tumwasorn, W. Kulwichit *(Bangkok, TH)*

Objectives: Tuberculous Pleuritis remains a major health problem worldwide. Laboratory diagnosis usually involves pleural fluid (PF) analysis and pleural tissue histopathology plus both specimens for culture and PCR. Diagnostic yields of PF PCR, however, is variable in different studies. Large volumes of specimens may give a better microbiologic diagnosis in certain conditions, as described in PF culture for *M.tuberculosis* and in PCR of vaginal discharge for *C.trachomatis*. Higher PF volumes may provide a better diagnostic yield of TB pleuritis.
Methods: A prospective observational study was conducted in patients with lymphocytic exudative pleural effusion at our university hospital from December 2004 to November 2005. Diagnosis of TB pleuritis is made by EITHER presence of pleural granuloma with or without acid fast bacilli plus a response to antituberculous therapy OR positive mycobacterial cultures of PF and/or pleural tissue. Patients with other final diagnoses served as a control group. Pleural fluids were divided in the laboratory into four different volumes of 1, 10, 50, and 150 ml before processing. The specimens were then centrifuged and the pellets produced were used for DNA extraction. Nested PCR using primers targeting IS6110 conserved among organisms in the TB complex group was performed. All standard PCR precautions were exercised. Presence of PCR inhibitors was tested in all specimens.
Results: 29 patients and 24 controls were enrolled (Table). In the TB group, 6, 8, 12, and 6 of 29 specimens of 1, 10, 50, and 150 ml showed positive PCR results (sensitivity of 20.69%, 27.59%, 41.38%, and 20.69%, respectively). All control samples revealed negative results in all volumes used. The 150-ml group with positive PCR also gave positive results in all other volumes. The 50-ml group with positive PCR covered positive results in all other groups plus 4 additional cases. Processing of the 150-ml specimens occasionally produced large, sticky pellets potentially affecting the DNA quality. PCR inhibitors were detected in three specimens (from different patients) of 10, 50, and 150 ml each in the controls.

CASES (No)	PCR results	Amount of pleural fluid (ml)			
		1 ml	10 ml	50 ml	150 ml
TB pleuritis (29)	Positive	6	8	12	6
	Negative	23	21	17	23
Controls (24)	Positive	0	0	0	0
	Negative	24	23*	23*	23*

*one uninterpretable specimen each due to PCR inhibitors

Conclusion: Higher volumes of pleural fluid increase a diagnostic sensitivity of tuberculous pleuritis by PCR to a certain extent. Too-large volumes, however, are unsuitable, probably due to presence of interfering proteins and other substances. Appropriate pleural fluid volumes should be assessed and verified in each laboratory.

P1112

The value of the QuantiFERON-Gold TB test in the management of patients with suspected active tuberculosis

J. Democratis, S. Bailey, M. Barer, N. Pereira, I. Stephenson, K.G. Nicholson, M.J. Wiselka, J. Democratis *(Leicester, UK)*

Objectives: The QuantiFERON-Gold TB Test detects interferon gamma production when whole blood is incubated with purified mycobacterial antigens. The test is able to detect latent and active TB, but has not been fully evaluated in patients presenting with suspected active TB. This study investigated the use of the test after its introduction in a large Regional Teaching Hospital in the UK with a high incidence of tuberculosis infection.
Methods: The QuantiFERON-Gold TB test was introduced into routine clinical practise in our centre in early 2005 and patient details were prospectively entered into a clinical database. This study reviewed the patient demographics, indications for testing, turnaround time for results and the additional clinical value of the test. The QuantiFERON-Gold TB Test was performed in all patients attending the Department of Infection with suspected active tuberculosis and results were compared with conventional tests including tuberculin skin testing (TST), microscopy, culture, histology and radiology.

Results: During the study period (Jan-Sept 2005) 270 tests were requested. The majority of requests were for suspected active tuberculosis in patients attending the Department of Infection, including HIV-positive and other immunosuppressed patients. The number of patients receiving TST's declined as the QuantiFERON-Gold TB test was introduced. Over 40% of patients had confirmed tuberculosis infection and there was generally good agreement between the clinical findings and the QuantiFERON-TB test. However 4 cases of disseminated TB in immunocompetant patients had negative tests, the possible reasons for this finding will be discussed. In 18% of cases the test was considered to have led to a change in clinical management with anti-TB therapy either commenced or discontinued as a direct result of the QuantiFERON-Gold test result.
Conclusions: The QuantiFERON-Gold TB test was of considerable value in the management of patients with suspected TB and led to a change in treatment in a significant number of patients. The results must be interpreted in conjunction with all other available information, but interferon-gamma tests are likely to play an increasingly important role in the management of patients with suspected TB and will replace the use of tuberculin skin testing in our institution.

P1113

Prospective MIRU-VNTR typing of *Mycobacterium tuberculosis* isolates: initial epidemiological analysis of isolates typed in the Midlands from 2003–2005

J.T. Evans, E.G. Smith, S. Gardiner, J. Mowbray, H. Mossop, B. Olowokure, P. Monk, P.M. Hawkey *(Birmingham, Leicester, UK)*

Objectives: From 2003–2005, all 1,952 *M.tuberculosis* isolates received at Midlands Regional Centre for Mycobacteriology were prospectively typed by MIRU-VNTR. The aim was to prospectively detect links between patients with active TB disease in support of conventional methods. The initial analysis of epidemiological data is presented in this study.
Methods: The 3 ETR and 12 MIRU-VNTR loci were amplified for each isolate and 5 μL of each amplicon loaded onto a WAVE® DNA Fragment Analysis System and analysed at 0.9 ml/min for 8.2-min (20–900 bp) at 50 °C. Basic epidemiologic data was gathered from specimen forms and analysed in comparison to MIRU-VNTR typing results using BioNumerics version 4.01.
Results: The clustering rate in ten strategic health regions ranged from 41–57%, with an average of 53%. Clustered MIRU-VNTR profiles were present in from 1 to 9 regions with any MIRU-VNTR clustered profile present in a median of 3 different regions. The median patient age was 35 years old with a range of 0 – 97. The most prevalent age group was 20–34 years old with 767 (39 %) patients, with 399 isolates (20%) from patients over 60 years old. 1,304 (67%) isolates were respiratory specimens and 636 were non-respiratory (33%) with 12 unknown (1%). The most prevalent MIRU-VNTR Profile was VNTR 32333 MIRU 224325153314 with 70 isolates (3.6%) detected in 14 laboratories and 7 health regions across the Midlands. 30 isolates (1.5%) were rifampicin resistant, 106 isolates (5.4%) were isoniazid resistant, and 23 patients (1.2%) were infected with MDR-TB. 8 MDR-TB isolates were grouped into 3 clusters by MIRU-VNTR typing, representing a 4.3% transmission rate of MDR-TB, thus suggesting that MDR-TB is not transmitted in the Midlands.
Conclusion: The relatively high transmission rate, proportion of respiratory cases and relatively low median age of cases, suggests that the emergence of TB in the Midlands may be due to newly acquired infections and not to disease reactivation.

P1114

Evaluation of clinical utility on direct detection of MTBC on clinical samples by BD Protec ET System

J.S. Lin, Y.Y. Yu, C.Y. Lin *(Changhua, TW)*

Objectives: The clinical utility and outcome of molecular diagnosis directly on clinical samples becomes popular and the performance needs to be monitored periodically. This study aimed to assess the performance of BD Protec ET system (Protec) in direct detection of MTBC in pulmonary and non-pulmonary samples in a tertially care medical centre.
Methods: From 2004 to October 2005, we retrospectively reviewed laboratory results of pulmonary and non-pulmonary samples that were referred for direct detection of MTBC by Protec. d-TB probes (BD) were used to amplify a DNA fragment, i.e., IS6110, within MTBC gene. The positive rate of MTBC identified by Protec were compared with the one which was determined by a rapid culture system (BD Bactec MGIT 960) followed by molecular identification using in-house multiplex PCR.
Results: A total of 424 clinical samples consisting of 195 pulmonary (46.0%)and 229 non-pulmonary (54.0%) specimens were recruited for this study. Non-pulmonary samples were obtained from ascites (16), blood (51), CSF (59), pleural effusion (32), gastric juice (12), synovial fluid (3) and urine (48), pus (1) and others (7). Only 134 (31.6%) of 424 samples were also requested for MTBC culture simultaneously. The positive rate for MTBC by Protec was 19.8% (84/424), whereas they were 24.6% (48/195) in pulmonary and 15.7% (36/229) in non-pulmonary samples, respectively. Among 134 samples submitted to Protec as well as culture, the positive rate for MTBC by Protec was 21.6% (29/134) as compared with 10.4% (14/134) by culture. This pattern was also shown in a subgroup analysis when Protec was compared with culture, i.e., 22.2% vs. 11.1% in pulmonary samples and 21.1% vs. 9.9% in non-pulmonary samples. Only 11 (8.2%) of 134 samples showed positive for MTBC by both Protec and culture (7 in pulmonary and 4 in non-pulmonary subgroups). Three samples positive for MTBC by culture (3/14, 21.4%) were negative by Protec, whereas 18 (18/120, 15.0%) samples positive for MTBC by Protec was negative by culture. The concordance rate (both positive and both negative) between Protec ET System and culture was 84.3%.
Conclusion: A lower submission rate (31.6%) for MTBC culture along with direct detection was noted in clinical behaviour. Although the concordance rate of MTBC identification between direct detection and culture seems acceptable, the high false negative rate of Protec reminds of the importance of simultaneous culture of MTBC in direct detection on clinical samples.

P1115

Clonal diversity of *Mycobacterium tuberculosis* in sputum specimen from individual patients of pulmonary tuberculosis

C. Shiohira, Y. Bhusal, N. Yamane *(Okinawa, JP)*

Objectives: The isolation of *Mycobacterium tuberculosis* from sputum specimen is the principal tool to make a definite

diagnosis on tuberculosis patients. Each isolate is generally considered to be monoclonal by nature and is directly employed for phenotypic and genotypic characterization. However, the properties of individual colonies from a single specimen largely remain unknown. In the present study, we determined clonal diversity of *M. tuberculosis* isolates from individual patients of pulmonary tuberculosis by using antimycobacterial susceptibility testing and IS6110 restriction fragment length polymorphism (RFLP).
Methods: A total of nine patients were included in the study. Five were newly diagnosed and four were undergoing chemotherapy. The sputum specimen was treated with NALC-NaOH, then plated on Middlebrook 7H11 agar plates. After incubation, 10 to 15 colonies were selected and subcultured. For individual colonies, antimycobacterial susceptibilities against eight agents were determined by broth microdilution test and DNA fingerprinting patterns were analysed using IS6110 RFLP as well as spoligotyping.
Results: There were significant differences among the individual colonies from a single isolate on susceptibility testing. Three isolates, including one newly diagnosed, were mixtures of susceptible and resistant colonies against streptomycin, isoniazid and/or ethambutol. Whereas, RFLP revealed that five isolates, including three newly diagnosed, contained heterogeneous colonies on RFLP. However, we found no significant correlation between RFLP patterns and antimycobacterial susceptibilities.
Conclusions: The study indicates that, in some patients of pulmonary tuberculosis, clonal diversity on phenotypic and genotypic characterization is present. The diversity of the isolates from patients treated with antimycobacterial agents were likely, because of the induction of drug resistance through the accumulation of spontaneous mutations. However, polyclonal infection with *M.tuberculosis* in patients not previously treated is often the case. Possible infection with polyclonal strains of *M.tuberculosis* and heterogeneity in drug susceptibilities during the single episode of tuberculosis is an important consideration from epidemiological and therapeutic aspects.

P1116

Agreement of QuantiFERON-TB Gold In-Tube and T Spot-TB in HIV-infected patients from Sub-Saharan Africa in Switzerland

M. Reichmuth, C. Thurnherr, F. Suter-Riniker, P. Vernazza, H. Furrer, T. Bodmer for the Swiss HIV Cohort Study

Objectives: HIV co-infection is the most important risk factor for the progression of latent tuberculosis infection (LTBI) to overt tuberculosis disease. Diagnosis of LTBI traditionally relies on tuberculin skin test (TST) results. Specificity of TST is confounded by previous BCG vaccination resulting in false-positive TST results. Sensitivity is reduced in patients with severe immunosuppression with cutaneous anergy, resulting in false-negative TST results. Novel interferon-gamma release assays (IGRA) utilize *M.tuberculosis* specific antigens providing results not confounded by BCG-vaccination. The goal of our study was to compare the performance of two commercially available IGRA in patients of the Swiss HIV Cohort Study (SHCS).
Patients: From November 2004 to March 2005, 34 HIV-positive immigrants (14 males, 20 females) originating from Sub-Saharan Africa were prospectively studied for LTBI during their regular clinical follow-up visits as patients of the SHCS. The respective CD4+ cell counts were: >500/mm^3 in 7 pts; 200–499/mm^3 in 23 pts; and <200/mm^3 in 2 pts. The median CD4+ cell count was 375/mm^3 (range, 91–922/mm^3).
Methods: Two IGRA test systems were used according to the respective kit instructions: QuantiFERON-TB Gold In-Tube (QFT; Cellestis Ltd., Carnegie, Australia) and T Spot-TB (Oxford Immunotec Ltd., Oxford, UK). For the latter, peripheral blood mononuclear cells were separated from 8 ml citrate-blood (Vacutainer Cell Preparation Tubes, Becton Dickinson, Franklin Lakes, USA) within two hours after phlebotomy. For QFT, INF-gamma release into the plasma was detected by EIA provided by the manufacturer; for T Spot-TB, INF-gamma spot forming cells were counted by the AID Elispot Reader System (AID GmbH, Strassberg, Germany).
Results: A total of 6/34 (17.6%) and 7/34 (20.6%) patients had positive test results with QFT and T Spot-TB, respectively. The observed agreement between both assays was 97% (kappa = 0.91, 95% confidence limits 0.72–1.00).
Conclusions: The very good agreement between QFT and T Spot-TB suggests that both assays are equivalent for the diagnosis of LTBI in HIV patients.

P1117

Role of serologic tests (IgG, IgA, IgM antibodies against A60 mycobacterial antigen) in diagnosis of tuberculosis

H. Emadi, M. Rasoolinejad, O. Zarghom, S. Siroos Jafari, Z. Ahmadinejad, Z. Abdi Liae, S. Davoudi, M. Hassibi, A. Soodbakhsh *(Tehran, IR)*

Objectives: The identification of acid-fast bacilli (AFB) in sputum or tissue is the definite diagnosis of tuberculosis. However, this method of diagnosis is restricted by certain limitations. The serologic diagnosis of tuberculosis has been used for a long time. The aim of this study was to determine the sensitivity and, specificity of Antigen 60 (A60) IgG, IgA, IgM findings in patients with tuberculous and to assess its application in the serologic diagnosis of pulmonary tuberculosis.
Methods: ELISA test based on mycobacterial A60 was used to measure specific IgA, IgM and IgG antibodies in the sera of 127 cases of tuberculosis, 13 cases of tuberculous HIV positive patients and 95 controls (46 were healthy volunteers and 49 other controls were patients with chronic diseases of different types.) The data of A60 IgG using enzyme-linked immunosorbent assay (ELISA), chest radiography, tuberculosis culture and pathology were obtained. The cutoff value of A60 IgG, IgA and IgM were chosen according to a receiver operating characteristic (ROC) analysis. The sensitivity, specificity and positive likelihood ratio were determined.
Results: The mean levels of IgG, IgA and IgM was significantly higher in patients of pulmonary tuberculosis when compared with control groups.Sensitivity of the IgG test was 54.3%, while the specificity was 84.2%. The IgA test showed a sensitivity of 670.1% with a specificity of 80%. Combination of the IgG and IgA tests showed a sensitivity of 45.7% and a specificity of 94.7% and the positive likelihood ratio was 8.62. In this study the chosen cutoff value of IgG IgA, IgM set at 285,265 and 0.9 ELISA units.
Conclusions: Our study shows a good specificity (94.7%) and a reasonable positive likelihood ratio (8.62) of the test when combined IgA and IgG with new cutoff points are considered, in the diagnosis of adult's tuberculosis. The combined use of both tests allows an increase in diagnosis of tuberculosis.

P1118

Implementing DOTS in settings with high prevalence of drug-resistant tuberculosis

Y. Balabanova, I. Fedorin, S. Zakharova, V. Nikolayevskyy, R. Atun, R. Coker, F. Drobniewski *(London, UK; Samara, RU)*

Only a few DOTS programmes have been implemented in the Russian Federation, one of 22 high burden countries.
Objective: Describe DOTS cohorts recruited in Samara Oblast (pilot Oblast) and assess the outcomes.
Methods: DOTS cohorts were recruited from April 2002 till September 2004 across civil and prison sector. Standard technical documentation and diagnostic/treatment protocols have been employed. All received cultures underwent subsequent epidemiological molecular typing using IS6110 insertions detection in the dnaA-dnaN intergenic region.
Results: 2093 patients were recruited. Only 29.3% of new cases were smear-positive in total and 42.9% of all new cases had bacteriologically confirmed TB. Drug resistant rates including multi-drug resistance were substantial with significantly higher rates in prison patients versus civil patients (Table 1). Approximately half of all isolated strains (51.7%; 371/717) belonged to the Beijing family with a significantly higher proportion isolated from prisoners compare to civilians (60.9%; 117/192 vs 48.4%; 254/525; RR1.3 (95%CI 1.1–1.5). In total 75.3% (592/786) of patients successfully completed the course of TB therapy while 7.3% (57/786) failed or defaulted, the rate being significantly higher among smear-positive patients compared to smear-negative (RR 3.3; 95%CI 2.0–5.4). The death rate was 3.6 % (28/786); a significantly higher proportion (11.3%) of smear-positive cases dying versus smear-negative (RR 11.2; 95% CI 4.6–27.2).

New cases	Prison (N-180)	Civil (N-569)	RR and 95%CI
Inh	79 (43.9%)	117 (20.6%)	2.1 (1.7-2.7)
Rif	70 (38.9%)	97 (17.0%)	2.3 (1.8-3.0)
MDR TB	68 (37.8%)	77 (13.5%)	2.8 (2.1-3.7)
S	93 (51.7%)	77 (13.5%)	3.8 (3.0-4.9)
E	45 (25.0%)	49 (8.6%)	2.9 (2.0-4.2)

Conclusions: Lower case detection rate can be addressed by changing the national policy of radiological screening; prioritarisation of bacteriology in diagnostic, monitoring and measurement of cure of TB as well as and further improvement of laboratory services. The rate of successful treatment was below the 85% WHO threshold but slightly higher than reported from other DOTS pilot regions in the former Soviet Union. High prevalence of primary drug resistance (associated with Beijing strain) proves active transmission. It is vital to improve patients' adherence to therapy to reduce acquired resistance and improve institutional infection control measures to prevent transmission. Introduction of rapid techniques of drug resistance identification to allow early resistance detection needs to be considered.

P1119

Multidrug-resistant tuberculosis: guideline tailoring for immigrants

T. Bregenzer, F. Schmid, C. Ebnöther *(Aarau, CH)*

Background: Guidelines recommend empiric therapy (ET) for tuberculosis (TB) with four first-line drugs. Patients with multidrug-resistant (MDR) TB are at risk for treatment failure and acquiring additional resistance. MDR TB has to be considered if standard regimen fails. MDR is prevalent in up to 14% in countries of the former Soviet Union.
Case report: We report on a case of a 20-year-old Mongolian woman who presented with night sweats and abdominal pain in May 2004. She travelled to Switzerland in March 2004. Microscopic sputum examination, culture and PCR were negative. Chest CT-scan showed multiple nodules in both lungs and the mediastinum. Bronchial biopsies showed necrotisizing granulomas. ET was started with rifampicin (RIF), isoniazid (INH), pyrazinamid (PZA), and ethambutol (EMB). Fever persisted and the patient's condition deteriorated. CT-scan showed progressive disease with multiple abscesses and vertebral osteolytic lesions. Cultures were repeated and ET was switched to linezolid, moxifloxacin and amikacin in August 2004. Resistance tests (October 2004) showed resistance for INH, RIF, EMB, SM, PZA and Cycloserin. It was sensitive to fluorochinolons, linezolid, amikacin, PAS, and capreomycin. Sequential resistance tests confirmed that PZA resistance was acquired during the first ET. In Dec 2004 a simplified oral regimen was started with linezolid and moxifloxacin. The further course was complicated by severe peripheral axonal degeneration probably due to linezolid (December 2004) and catheter related *P. aeruginosa* sepsis (March 2005) after replacing linezolid by amikacin. As of May 2005 the patient was in good general condition. Therapy was discontinued after 12 months, august 2005.
Conclusions: Standard treatment with four or five first-line agents may fail in patients who immigrate from regions with a high prevalence of MDR TB and may induce further resistance. If ET is necessary in a patient with suspected MDR TB local resistance data should be taken into consideration. The risk of treatment failure with ET has to be weighed against the risks associated with delayed treatment while waiting for resistance results.

P1120

Rapid drug sensitivity testing of *M. tuberculosis* based on culture on a porous ceramic support

C. Ingham, A. Ben Ayad, R. Hummelink, B. Mulder *(Den Bosch, Enschede, NL)*

Objective: The growth of *M. tuberculosis* rate-limiting in many diagnostic procedures including drug sensitivity testing (DST). Attempts to speed up DST for this organism have included the detection of microbial metabolism and the optical imaging of microcolonies. Currently, there is no molecular method for determining the full drug resistance pattern required to guide effective treatment regimes. The objective of this work was to use a highly porous ceramic (Anopore) as a culture support to facilitate the rapid detection of *M. tuberculosis* growth, and to apply this capability to DST. This is a novel methodology, one that exploits the high porosity and excellent culture and imaging properties of Anopore.
Methods: Sterile anopore strips were placed upon Middlebrook 7H10 agar plates and inoculated with *M. tuberculosis* stains 445 (RIFS, INHS) and 473 (RIFS, INHR). The plates were incubated in a CO2 incubator at 37 degrees C. Cultures were then killed by placing on filter paper disks saturated with methanol or by heat treatment. This was followed by the transfer of the Anopore to a microscope slide covered with a film of solidified agarose containing 20 μM Syto16 dye for 45 min. Staining of cells on the Anopore surface was accomplished with minimal disruption of the microcolonies on the surface. Microcolonies were then imaged directly using an Olympus BX41 epifluorescence microscope equipped with Fluorotar lenses. Image capture used an 8-bit Kappa CCD camera and BMP files of images were analysed using ImageJ software.

Results: Colonies of *M. tuberculosis* were visible with 14 days incubation on Anopore strips placed on Middlebrook 7H10 agar plates and were similar in size and morphology to culture directly on the same agar base. Individual cells and microcolonies could be detected after Syto 16 staining. After only 2–3 days culture the increase in microcolony area was found to be significant ($P < 0.001$, $n < 200$, 2-tailed Mann Whitney U-test) indicating growth had occurred. DST testing against Isoniazid and Rifampin is in progress and the results will be shown.
Conclusions: Anopore is an effective culture support for *M. tuberculosis*. Imaging microcolonies *in situ* has been used to detect growth within a few days of inoculation and is now being applied to DST. The ability to kill, stain and image microcolonies *in situ* was facilitated by the porous, inert and rigid nature of Anopore.

P1121

Identification of the *Mycobacterium tuberculosis* subtype strongly associated with multidrug resistance within the Beijing family

V. Nikolayevskyy, T. Brown, Y. Balabanova, Y. Bazhora, A. Asmolov, F. Drobniewski *(London, UK; Odessa, UA)*

Background: Investigation of *M. tuberculosis* drug resistance rates, prevalence of certain genotypes and their association with mutations conferring drug resistance in Ukraine may contribute significantly to the development of appropriate interventions to combat converging TB and HIV epidemics.
Objectives: (i) to determine the degree to which isoniazid, rifampicin and multiple drug resistance (MDR) was associated with Beijing family isolates and subgroups within Beijing family; (ii) to identify molecular genotyping methods suitable for differentiation of isolates in populations where highly conserved genotypes dominate; (iii) to confirm our hypothesis that the prisons are major drivers of drug resistance.
Methods: Non-commercial reverse hybridization assay (macroarray) was used for detection of key mutations responsible for the development of resistance to rifampicin and isoniazid. Genotyping was done using multilocus VNTR typing using the panel of 12 MIRU and 3 ETR loci. Beijing strains were identified using spoligotyping.
Results: Total of 225 *M. tuberculosis* strains isolated from patients with radiologically and bacteriologically confirmed TB in Odessa and Nikolaev regions of the Southern Ukraine were investigated. MDR rates were significantly higher in former prison inmates compared to those never been in prisons (54.8% vs 27.3%, RR 2.01, 95% CI 1.35–2.97). Mutations in codons 528–533 of the rpoB gene and codon 315 of the katG gene dominated in drug resistant strains. The overall discriminatory ability of VNTR genotyping for the given group of Ukrainian isolates was higher than that provided by spoligotyping (HGDI 0.968 versus 0.878) with 68.6% of Beijing strains comprising two large clusters formed of 35 and 21 strains sharing the MIRU-ETR signatures 223325153533424 and 223325173533424 respectively. Isolates with the latter MIRU-ETR signature were more common among previously imprisoned patients. Prevalence of mutations conferring MDR was significantly higher in the subgroup of Beijing strains sharing VNTR signature 223325173533424 compared to other Beijing strains (71.4% vs 45.7%, RR 1.74, 95% CI 1.17–2.57).
Conclusions: Significantly higher prevalence of drug-resistant Beijing strains in former prison inmates suggests that prisons are major drivers of drug-resistant TB. Beijing genotype with VNTR signature 223325173533424 is strongly associated with drug resistance and may be responsible for rapid transmission of MDR TB in Ukraine.

P1122

Extrapulmonary tuberculosis in sector III of Zaragoza, Spain during 2000–2005

M.P. Macipe, A. Vitoria, J.A. Amiguet, A.I. López-Calleja, M.A. Arias, P. Crusells, J. Cuesta, C. Rubio *(Zaragoza, ES)*

Objectives: To analyze the evolution and the clinico-epidemiological characteristics of the cases of extrapulmonary tuberculosis with microbiological confirmation, in population belonging to the Sector III of Zaragoza, H.C.U."Lozano Blesa" between 2000–2005. To value by means of RFLP the relationship between the isolated strains and the different clinical presentations of the disease.
Material and methods: A retrospective study of all patients with extrapulmonary TBC was made (cases with positive culture to *M. tuberculosis*, in any location different to the pulmonary one) during the period to study. We revised the variables: Age, nationality, origin service, type specimen, clinic, HIV, sensibility to antituberculostatic drugs and evolution of the disease. We studied the RFLP carried out in the Faculty of Medicine in Zaragoza in all the isolated strains between June 2001 and June 2005, to establish the relationship between the found clusters and the different extrapulmonary locations.
Results: We isolated *M. tuberculosis*, in extrapulmonary locations, in 58 patients. The biggest number of cases (25%) was in the year 2004. The most frequent locations were: pleural TB, lymphnode TB and genitourinary TB. Only in 31% of the samples, the baciloscopy was positive. 84% of the strains grew in Lowestein-Jensen medium, and 91% made it in the MB/Bactec® means. Only 5 of them (8%) presented some type of resistance to antituberculostatic drugs: 3 resistant to isoniazid, 1 to isoniazid/estreptomicini and another one to isoniazid/etambuhtol. 15% of the patients were immigrants, most of them coming from Africa, in which any resistance type was not detected. Most of the patients were HIV negative or this serologic determination was ignored. In the 58 isolated strains, 8 of them were part of some cluster, from those tipificated among all the cases of pulmonary and extrapulmonary tuberculosis, diagnosed in the period of the study. In one of them, coming from pleural biopsy, we found resistance to isoniazid.
Conclusions: The extrapulmonary locations were the 22% of the patients' diagnosed of tuberculosis. These manifestations depend on factors associated to the host. There are not data to conclude that identical patterns of RFLP correspond to specific locations of the disease. *M. tuberculosis* tipification by using IS6110 has proved to be a valuable tool in epidemiological TBC studies. Cases of extrapulmonary TBC appear inside the clusters determined in the period to study.

P1123

Evaluation of tuberculin skin test and Quantiferon-TB gold for the identification of LTBI or active TB among irregular immigrants in Milan, Italy

G. Orlando, S. Merli, A. Valerio, L. Cordier, M. Bergonzi, A. Villa, A. Cargnel *(Milan, IT)*

Objective: Tuberculosis infection is increasing worldwide. Targeted testing to identify persons at high risk for TB who would benefit from treatment for latent TB infection (LTBI)

would include recent or irregular immigrants from developing countries. Tuberculin skin testing (TST), generally accepted for routine evaluation, does not differentiate between *M. tuberculosis* infections and BCG vaccination and, needing at least 2 accesses to health care facilities, has a reduced diagnostic power in critical settings. Here we report an interim analysis of a clinical trial in which TST and Quantiferon-TB Gold are evaluated for the identification of TB infections among irregular immigrants.
Methods: Consented immigrants observed in the Opera San Francesco outpatients department for any health problem underwent to TST evaluation (time 0 and 72 h) through injection of 0.1 mL of 5 TU PPD and to a blood puncture. TST was considered positive for an induration less than 10 mm (recent immigration) or greater than 15 mm (old immigration). Whole heparinized venous blood samples were analysed after 16–24 h incubation through Quantiferon-TB assay for cell mediated immune responses to *M. tuberculosis*-specific antigens (ESAT-6, CFP-10).
Results: Since July 2005, 121 (71 F, 50 M) immigrants have been enrolled; median age 37 years (IQR 29–45). Countries of origin were Latina America 53.7%, Eastern Europe 25.6%, Asia 10.7%, and Africa 9.9%. The patients arrived in Italy <1 year (29.4%), 1–3 years (47.9%), 3–5 years (9.2%) and >5 years (13.4%) before the test. The overall positivity to at least 1 test was 51.2%. TST were positive in 43 (35.5%), with 18 patients dropped out. Quantiferon-TB test resulted positive in 44 (36.36%). 103 coupled test are available: concordance of results were obtained in 68%. Discordance was characterized by 14.6% Quantiferon pos/TST neg results and 17.4% Quantiferon neg/TST pos results. In 4/18 dropped TST patients (22.2%) a positive Quantiferon TB test was identified.
Conclusions: This analysis underlines that recent immigration from developing countries and irregular immigration are factors related to increased risk of infection and progression to clinically active disease. TB infection control need targeted screening programs able to reach all susceptible populations. The very high prevalence of positive results in at least 1 test and of discordant data suggest the need for further analysis.
This work is supported by a grant from Provincia di Milano.

P1124

Tuberculosis epidemiology in a health care area of Santiago de Compostela (Galicia, NW Spain) from 1996 to 2004

V. Tñez, M.L. Pérez del Molino, P.A. Romero, M. Conde, F. Pardo *(Santiago de Compostela, ES)*

Objective: A Tuberculosis Control and Prevention Gallego Programme (PGPCT) has been conducted in Galicia, organized in Health Care Areas, attended in a Reference General Hospital by territories. There are seven Health Areas in Galicia, each one has a Tuberculosis Unit with: medical assistance, surveillance-control, and recording data of all cases of the area. The aim of this study is to analyze the Tuberculosis epidemiology changes during the period 1996–2004 in the Health Care Area of Santiago de Compostela (459.180 inhabitants).
Methods: An epidemiologic descriptive study of temporary inclination was conducted, the variables included are: localization, HIV co-infection and Directly Observed Therapy (DOT). Microbiological studies considered: auramine stain, growth in liquid medium (Middelbrook 7H9-ESPII) and solid Coletsos medium. We identified the strains with M tuberculosis complex AccuProbe®.
Results: During this period 2197 cases has been diagnosed, 1286 (58.5%) males and 911 (41.5%) females. The incidence rate descended from 72.79/100.000 in 1996 to 33.58/100.000 in 2004. The incidence for pulmonary disease decreased from 45.86/100.000 to 22.92/100.000 meanwhile the bacilliferous cases decreased from 26.70/100.000 to 16.44/100.000 from 1996 to 2004. The pulmonary location is 65.4%, 25.1% extrapulmonary and 9.5% pulmonary+extrapulmonary. In 1996 we had 119 bacilliferous patients, 145 positive cultures and 32 clinical diagnosis (no microbiological confirmation), while in 2004 there was 71 bacilliferous cases, 83 positive cultures and 9 clinical diagnosis. 3.7% of DOT were conducted in 1996 in comparison with 19.4% in 2004. Maximum incidence was detected between 15–44 years old. Cases with HIV coinfection has been reduced from 4.34% in 1996 to 2.1% in 2004.
Conclusions: 1. -Tuberculosis disease incidence in Santiago is higher than expected for its social and economic level, but it decreased since this programme has been established, probably due to the exhaustive follow up of the cases and DOTs implementation. 2. -A high incidence persists in younger groups, specially with pulmonary disease, although a decrease was observed. 3. -The percentage of HIV and tuberculosis coinfection is not determinant of the epidemiological characteristics of tuberculosis in Santiago.

P1125

Tuberculosis in paediatric and adult population in Athens, Greece (1994 – 2004)

S. Kanavaki, S. Karabela, A. Skroubellou, E. Varonou, S. Anagnostou, A. Raftopoulou, H. Moraitou, N. Spyridis, S. Nikolaou *(Athens, GR)*

Objectives: We studied retrospectively the new pediatric and adult TB cases diagnosed in the National Reference Laboratory for TB in Athens during the period 1994 – 2004.
Material & Methods: Pediatric TB cases were 77, while adult TB cases 4278. From children, 32 were <2 years, 21 were 2–5 years, 14 were 6–10 years and 10 were 11–16 years of age.
Results & Discussion:
• A peak in new TB cases was noticed in 200, for both populations studied. It is of great concern that the peak of pediatric and adult TB cases was noted in 2000, when migration reached the highest peak in Greece.
• In relation to resistance to the main first line antituberculus drugs, 15.5% of the TB isolates in pediatric population were resistant to Isoniazide (INH), 3.8% to Rifampicin (RIF), while 3.8% were multidrug resistant (INH+RIF). However, it is interesting that in adult population, resistance to INH was significantly lower 9.1%, resistance to RIF was 4.2% and multidrug resistance 3.5%. Concerning Streptomycin (SM), it is also interesting that 14.2% of pediatric TB isolates were resistant, in contrast to 2.5% of TB isolations in adult population studied. For having a satisfying explanation of these findings, it is necessary to further investigate young patients' medical files, in an attempt to associate contacts of young patients with infected adults, which is our purpose in the near future.

P1126

Screening of immigrants for tuberculosis in Greece: implications for health care needs

M. Matsagoura, M. Kompoti, A. Koutsovasili, A. Salta, A. Papaefstathiou, M. Matsagoura *(Thiva, GR)*

Immigrants from countries with a high prevalence of tuberculosis (TB) may allow a significant contribution to the increasing TB rate in the industrialized world. Screening for tuberculosis

enables new immigrants to receive important medical evaluation and treatment and provides useful surveillance data.
Objective: The aim of this study was to assess the prevalence of tuberculin skin testing (TST) positivity among immigrants newly arrived in Greece.
Materials and methods: To be allowed a residence permit, all immigrants in Greece are screened for TB. Screening procedure includes TST, a chest X-ray and thorough physical examination. All immigrants screened for TB during a two-month period in 2005 were included in our study. Demographical data were recorded for all subjects. Statistical analysis was performed using logistic regression and Pearsons chi-square test for categorical variables, Student's t-test for normally distributed and Mann-Whitney U test for skewed numerical variables. Statistical significance level was set at $p < 0.05$.
Results: A total of 237 immigrants were screened (172 males and 65 females). Mean age was 31.0 ± 10.6 years and mean time in Greece 2.8 ± 1.9 years. One-hundred twenty-one immigrants originated from Europe (51%), 109 (46%) from Asia and 7 (3%) from Africa. TST was positive in 41 subjects (17.3%), 28 males (16.2%) and 13 females (20%), $p = 0.725$. There was no significant difference in the prevalence of TST positivity in respect to origin. Among 86 subjects who developed a detectable skin induration, the presence of BCG scar was not associated with a greater TST reaction size (10.6 ± 6.7 mm vs. 11.6 ± 6.1 mm in subjects with and with no BCG scar, respectively, $p = 0.552$).
Conclusions: The prevalence of positive TST among immigrants to Greece is not negligible. A BCG vaccination in the early childhood must not substantially alter the interpretation of TST in adults. Given the low risk of acquiring TBC after arrival in Greece, preventive therapy should be considered for all immigrants with positive skin test (TST), a clear chest X-ray and no recent exposure to a known source case of tuberculosis, after adjusting for the risk of adverse effects from chemoprophylactic agents.

P1127

Epidemiology of *Mycobacterium bovis* disease in Ferrol, Spain, 1991–2004

J.F. García Rodríguez, H. Ivarez Díaz, V. Trasancos Buitrago, M.V. Lorenzo García, A. Mariño Callejo, P. Sesma Sánchez *(Ferrol, ES)*

Objectives: To examine the current epidemiology of *Mycobacterium bovis* disease and to compare patient characteristics to those of patients with *M. tuberculosis* disease in our community.
Methods: Prospective study of surveillance, epidemiological, and treatment completion data for cases of tuberculosis in our Tuberculosis Unit between 1991 and 2004. Variables examined included sex, age, race, country of birth, main location of disease, drugs susceptibility, HIV infection status, initial medications, medical provider type, treatment completion and course of disease. Differences between *M. bovis* and *M. tuberculosis* patient characteristics and treatment completion rates were compared using the χ^2 test or the Fishers exact test. Medians were compared using the Mann–Whitney test. Differences were considered statistically significant at a level of $p < 0.05$.
Results: 787 cases of culture-positive tuberculosis were identified, of these 782 were *M. tuberculosis* and 5 (0,6%) were identified as M. *bovis*, (incidence 0.17 per 100,000 person-years). All the patients infected with *M. bovis* were HIV-negative adults, they were born in Spain, none had travelled outside of Spain and no links that might allow airborne person-to-person transmission of *M. bovis* were discovered among any of the patients. They were older (51.2 ± 14.3 vs 38.1 ± 19.1 years) and had more extra-pulmonary disease (80% vs 32.1%, $p < 0.05$) compared with *M. tuberculosis* patients. *M. bovis* isolates were resistant to pyrazinamide but there were not differences in resistence to isoniazid (0/5 vs 12/451) and rifampin (0/5 vs 1/450) compared with *M. tuberculosis*. The median treatment duration was approximately 3 months greater for *M. bovis* than for *M. tuberculosis* patients. Differences in frequency of Direct Observed Treatment (1/5 vs 37/781), treatment completion rates (Default 0/5 vs 42/776), toxic hepatitis (0/4 vs 49/770) and evolution (Death by TB 1/5 vs 21/782; Relapse 0/4 vs 9/771) was no significant. Mean follow-up was 26.7 ± 25.9 months (range, 1–174; person-months, 21055).
Conclusion: The predominant clinical presentation was consistent with progression from latent TB infection. No person-to-person airborne transmission was identified. *M. bovis* infection is frequently of extra-pulmonary localization. Duration of treatment tended to be longer for patients infected with *M. bovis* and completion rates and evolution were comparable to those of patients infected with *M. tuberculosis*.

P1128

Tuberculosis as re-emerging disease in Italy. Which correlations with HIV infection and other risk factors, comparing native residents and foreign immigrants?

R. Manfredi, S. Sabbatani, G. Legnani, F. Chiodo *(Bologna, IT)*

Background: Tuberculosis (T) is borne by increasing morbidity and mortality rates, due to changes of epidemiologic scenario, and diffusion of resistant strains. The recent, profound modifications occurred among predisposing factors (increase mean patient [p] age, concurrent diseases, jatrogenic immunosuppression, alcoholism, drug use, migration, and HIV pandemic), played a key role in this process.
Methods: Among the 128 consecutive p hospitalized due to T since 1996, we compared the 77 p from Italy with the 51 immigrants from extra-European countries, in relation of a number of risk factors, including HIV infection.
Results: Compared with immigrants, native Italian p had a higher frequency of HIV-AIDS (32.4%; $p < 0.001$), and a prodominant pleuro-pulmonary involvement versus lymph-node and/or disseminated T among HIV-infected p versus non-HIV-infected ones ($p < 0.01$). Moreover, Italians had a greater mean age ($p < 0.001$), and an increased frequency and a broader spectrum of predisposing conditions (positive history, chronic lung, heart, liver, kidney disease, diabetes mellitus, malignancies, and collagen vascular disease; $p < 0.03$), while foreigners had a lower frequency of more generic supporting factors (low income, economic-social problems, cigarette smoke, and alcohol-drug abuse; $p < 0.03$ versus Italians). Our decade experience shed light on two different patterns of T. Local p are predominatly represented by elderly with frequent concurrent disorders and specific T risk factors, a more frequent HIV infection, and a predominant involvement of sites other than pulmonary ones, while immigrants are represented by otherwise healthy younger p, who develop prevalent lung localizations.
Conclusions: The clinicians awareness of T needs further attention, in order to obtain a rapid diagnosis and treatment, and reduce transmission risks. The progressive integration of immigrants with local population may lead to increased risks of T dissemination, especially among the local, more vulnerable and older population.

P1129

A randomised trial of a short course with isoniazid plus rifampin in front of isoniazid during six months in latent tuberculosis infection

D. Vañó, M.P. Geijo, C. Rosa, A. Molina, M. Pérez, J.F. Dimas *(Cuenca, ES)*

Objective: To evaluate compliance, side effects and the efficacy of a short course of chemoprophylaxis for tuberculosis with isoniazid plus rifampin during 3 months, compared with a course with isoniazid during 6 months.
Patients and methods: Prospective, comparative, randomized and open study of patients with the suitable criteria for chemoprophylaxis, in accordance with the guidelines of the Centres for Disease Control of 1990. HIV positive patients were excluded. Patients were divided into 2 groups: group I received isoniazid (300 mg per day) plus rifampin (600 mg per day) for 3 months, and group II received isoniazid (300 mg per day) for 6 months. We realised clinical and analytical follow-up during the period of treatment.
Results: 83 patients were included, 36 in the group I and 47 in the group II. Both groups were comparable at base level. It was hepatotoxicity in the 52.8 % of patients of group I in front of the 21.3 % in the group II ($p < 0.01$). There were no significative statistical differences in serious hepatotoxicity (8.3% vs 2.3%), digestive toxicity (5.6% vs 14.9%) or cutaneous toxicity (2.8% vs 2.1%). Chemoprophylaxis was retired before the end of the treatment in 5 patients of the group I and in 4 patients of the group II. The average follow-up of the patients was 52.14 months. No case of tuberculosis has been detected up to the moment.
Conclusions: A short course with isoniazid plus rifampin during 3 months is equal of effectively that the standard course with isoniazid during 6 months in the latent tuberculosis infection. The toxicity in short course is lower, probably in the minor time of exposition.

P1130

Mycobacterium sherrisii: a new opportunistic agent in HIV-infected patients?

P. Loulergue, F. Lamontagne, V. Vincent, A. Rossier, G. Pialoux *(Paris, FR)*

Background: Various atypical mycobacteriae have been described to cause disease in HIV- infected patients. We report the first case of a disseminated infection with *Mycobacterium sherrisii* in an advanced immunocompromised patient.
Case report: A 53 year old Causasian male, was referred to our hospital in May 2005. A fever of unknown origin and a generalized weakness have been noticed for several months. The patient was tested positive for HIV infection in September 2003 during an hospitalisation for pulmonary tuberculosis. In February 2005, an antiretroviral therapy was started with nevirapine, lamivudine and stavudine. A relapse of tuberculosis was suspected and treated with rifampicin, isoniazid, ethambutol and pyrazinamid.On admission, his CD4 cell count was 19.106/ml. Six to nineteen days later five sputum samples, one peritoneal effusion sample and two blood cultures grew positive with atypical mycobacteria. This bacteria was thought to be *M. simiae*. Treatment was changed for clarithromycin, rifabutin and ethambutol, without clinical improvement neither microbiological control (another blood culture was positive). The final identification revealed *M. sherrisii* after two months. The drugs susceptibilities were tested by BACTEC method and showed sensibility to clarithromycin, ethambutol, rifabutin, intermediate sensibility to amikacin and resistance to ciprofloxacin. Eventually, we added amikacin and moxifloxacin with a relative efficacy (general improvement, negativation of bacterial samples).
Discussion: *M. sherrisii* is a recently described mycobacteria, phylogenetically related to *M. simiae*. Diagnosis has been confirmed by gene sequencing of 16S rRNA and hsp65, and DNA-DNA hybridization. The only clinical case reported before was a HIV-infected african male with a pulmonary injury. A combination of clarithromycin, rifabutin, ethambutol, moxifloxacin and amikacin showed clinical and microbiological efficacy in our case but other combinations were ineffective despite of *in vitro* sensitivity.
Conclusion: Infection with *M. Sherrisii* can be considered as an emerging pathogen in HIV infected patients with poor immunologic status. The best treatment is not yet definite, and may not be strictly correlated to *in vitro* data.

P1131

Presence of mycobacteria in colon biopsies from patients with inflammatory bowel disease

I. Szpinda, A.T. Jorde, I. Olsen, B. Djønne, M. Vatn, T. Tønjum *(Oslo, NO)*

Objectives: Crohn's disease is a chronic condition with inflammation in the intestines. A strong correlation between the presence of *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease has previously been inferred, and a far higher incidence of *M. a. paratuberculosis* PCR positivity has been shown in the gut of Crohn's patients than in healthy people. It is, however, not clear if *M. a. paratuberculosis* has an etiological impact in the pathogenesis of Crohn's disease, or if it is just a coincidence in that Crohn's patients have an increased predisposition for colonisation by *M. a. paratuberculosis*. We extended this question to ask if there is an increased presence of mycobacteria in general in colon biopsies from patients with inflammatory bowel disease (IBD; Crohn's disease and ulcerous colitis) as compared to healthy people. To facilitate the mycobacterial detection we used cultivation in enriched media in combination with direct detection by PCR.
Methods: Biopsies were prospectively taken from inflammatory lesions from all sections of the colon from 200 patients submitted to colonoscopy with suspected IBD. One hundred healthy controls were also included. Patients were later grouped according to IBD status: Crohn's disease, ulcerous colitis, other condition or healthy colon. The biopsies were investigated for the presence of mycobacteria by *M. a. paratuberculosis*-specific IS900 PCR analysis and cultivation in the BACTEC MGIT 960 continuous culture system. The specimens were cultivated in mycobactin-enriched BBLTM Middlebrook 7H9 medium. Mycobacterial strains isolated were identified by species-specific hybridization and PCR, as well as 16S rDNA PCR sequencing and blast analysis.
Results: Among the samples from 60 out of the 200 patients to be included, only 1 patient was culture positive and 5 patients were IS900 PCR positive. These patients all had Crohn's disease. Mycobacteria were so far not detected in biopsies from the healthy controls. The results from the completed study will be presented.
Conclusion: Taken together the findings from this study will generate novel knowledge on the occurrence of mycobacteria in the human gut. This will extend the basis for defining mycobacteria have an etiological role in IBD, with special emphasis on Crohn's disease.

P1132

Prevalence of nontuberculous mycobacteria in a Belgian adult cystic fibrosis population

M. Develter, W. Laffut, L. Dupont, J. Verhaegen *(Leuven, BE)*

Objective: With the increased survival of cystic fibrosis (CF) patients, colonisation and infection with new respiratory pathogens have emerged. Among these are nontuberculous

mycobacteria (NTM). NTM are ubiquitous micro-organisms, but geographic differences in their prevalence exist [1]. The present study was done to determine the prevalence of NTM in the adult CF-patients attending the university hospital of Leuven (Belgium).

Methods: During an 18 month period (February 2004 until June 2005) sputum samples were obtained from 83 adult CF patients while attending the outpatient clinic. These samples were decontaminated using the NALC-NaOH method as described by the American Society for Microbiology [2]. The Mycobacteria Growth Indicator Tube (MGIT) (Becton Dickinson, USA) was used as a culture medium with incubation for 6 weeks in BACTEC MGIT 960 (Becton Dickinson, USA). In the case of overgrowth with micro-organisms other than mycobacteria, a second decontamination following the method described by Bange et al. [3] was performed on samples indicated by the clinician.

Results: Of 83 patients, 363 samples were obtained (1–12 samples/patient). For 46 patients, no growth of all samples (146) was seen. The remaining 37 patients had a mixture of no growth (128 samples) and overgrowth (89 samples), mostly with Gram negative organisms (60%), a minority with Gram positive organisms (30%) and a mixture of both (10%). On 17 (for 15 patients) of the overgrown samples secondary decontamination was performed with 2 samples remaining contaminated. There were no grown cultures with NTM.

Conclusion: NTM were not recovered from the airways of Belgian adult CF-patients despite secondary decontamination procedures.

References:

1. Olivier KN, Weber DJ, Lee JH, Handler A, Tudor G, Molina PL, Tomashefski J, Knowles MR. Nontuberculous mycobacteria in cystic fibrosis study group. Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. Am J Respir Crit Care Med 2003; 167(6): 828–834.

2. Pfyffer GE, Brown-Elliott BA, Wallace RJ. Mycobacterium: general characteristics, isolation and staining procedures. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. Manual of clinical microbiology. 8th edn. Washington: ASM Press, 2003: 532–559.

3. Bange FC, Böttger EC. Improved decontamination method for recovering mycobacteria from patients with cystic fibrosis. Eur J Clin Microbiol Infect Dis 2002; 21: 546–548.

P1133

Tuberculous empyema necessitatis involving chest wall and retroperitoneum

O.K. Aribas, E. Turk-Aribas, I. Erayman, F. Kanat, A. Ozturk *(Konya, TR)*

Empyema necessitatis (EN) is a rarely seen infection of soft tissue, which is formed by the spontaneous drainage of empyema through the parietal pleura. Although the most common site is subcutaneous tissue of the chest wall, it may include esophagus, breast, retroperitoneum, pericardium, groin and vertebral column. We present a patient with ES of chest wall and retroperitoneal space with psoas abscess complicated by untreated tuberculous empyema. A 21-year-old male patient was admitted with two months history of two growing masses on the left posterolateral chest wall and left lumber area following a left chest pain. Physical examination revealed fluctuating, 6 cm diameter, fixed, painful two swellings and diminished pulmonary sounds on the left hemithorax. Chest x-ray disclosed disclosure of the left costadiaphragmatic sinus and narrowing of the left intercostals spaces. Chest CT scan revealed a subcutaneous, 12×6×8 cm in diameter, loculated collection of high density fluid on the left lateral chest associated with pleural space and a multiloculated, 15×12×8 cm in diameter fluid collection between paraspinal musculature and lying into the psoas muscle in the retroperitoneum. Thoracenthesis revealed an empyematous fluid with negative nonspecific culture and the culture of aspiration of the thoracic wall mass was also negative for any nonspecific bacilli. Laboratory examination was normal except a mild leucocytosis and an increased ESR of 136 mm/hour. Left thoracotomy was performed with a diagnosis of ES. Purulent collections in the pleural space and in the abscess formations within thoracic wall and retroperitoneum were aspirated and later the patient underwent resection of the destructed tissues and left pleural decortication. The procedure was completed with a placement of the drainage tubes into the pleural, abdominal wall and retroperitoneal spaces. Histopathological examination of the resected tissues and pleura confirmed granulomatous inflammation with caseous necrosis. INH, RIF, EMB and PZA were given to the patient for two months and the treatment was continued for additional 7 months with INH and RIF. After 9 months of therapy the patient was in good health. EN is a well-known, however, extremely rare complication of the tuberculous empyema. Although the morbidity and mortality were high It is possible to treat with appropriate surgical debridement, drainage and antituberculous treatment without sequelas.

P1134

Increasing incidence of *Mycobacterium xenopi* in Zaragoza, Spain: an emerging pathogen or a product of improved laboratory methods?

S. Terraza, J.A. Amiguet, A. Vitoria, M.A. Lezcano, C. Ramos, P. Arazo, J. Cuesta *(Zaragoza, ES)*

Objectives: To investigate the clinical significance of *M. xenopi* isolation and explore the clinical spectrum of *M. xenopi* disease in Zaragoza, Spain.

Methods: A retrospective study was carried out between January 1990 and December 1999 in Miguel Servet Hospital, Clínico Lozano Blesa Hospital, and Royo Villanova Hospital. Diagnosis of the disease was performed according to American Thoracic Society criteria.

Results: The patient charts of 96 patients in which an isolate of *M. xenopi* had been recorded were reviewed. We recovered 40 *M. xenopi* isolates from 1990 to 1994, and 56 isolates from 1995 to 1999. We considered 10 patients to be suffering from disease caused by *M. xenopi*. The number decreased for the second half of the study period (7 vs. 3 cases). Pulmonary disease was the most frequent clinical presentation (90%). One patient had clinical *M. xenopi* disseminated disease during this period. Eighty percent of all patients with disease were male. The mean age was 39.5 year. The most common risk factors associated with disease due to *M. xenopi* were smoking (90%), infected with the human immunodeficiency virus (HIV) (40%), and chronic obstructive pulmonary disease (30%). The mean CD4 cell count of the HIV-infected patients at diagnosis was 48 x 106/L. The most frequent clinical features were the respiratory symptoms (100%), followed by fever (60%), and constitutional symptoms (30%). Chest radiographs revealed cavitating pulmonary disease in 50% of all patients, and nodular disease in 30 %. The incidence of unilateral disease was 60%. The most common treatment regimen was isoniazid, rifampicin, and ethambutol, and the average duration was 10 months. With treatment, all cases of non-HIV-associated disease had favourable outcomes, but those of HIV-associated pulmonary and disseminated disease were poor (50%).

Conclusion: The increase in *M. xenopi* isolates noted in the three main hospital in Zaragoza (Spain) was due to changes in culture

technique, and a more sensitive laboratory isolation technique, rather than a true increase in clinical disease. Chronic pulmonary disease is the most common clinical manifestation of *M. xenopi* in the nonimmunocompromised patient, but in HIV-infected patients causes both disseminated and pulmonary disease.

P1135

Mycobacterium chelonae and continuous ambulatory peritoneal dialysis associated peritonitis. An unrecognised entity?

I. Gupta, P. Cockwell, I. Das *(Birmingham, UK)*

Introduction: Continuous ambulatory peritoneal dialysis (CAPD) is the initial treatment for 30–40% of patients with end-stage renal disease (ESRD) in the UK. Peritonitis is the major complication and is a frequent indication for discontinuation of CAPD. Mycobacteria are rarely reported from such peritonitis.
Objective: To describe an unusual case of CAPD peritonitis due to Mycobacterium chelonae (*M. chelonae*).
Methods: University Hospital Birmingham is one of the UK's leading centres for renal dialysis and transplantation. Investigation for CAPD peritonitis includes microscopy of peritoneal dialysis (PD) fluid for white blood cell count and culture by inoculation into blood culture bottles which is monitored by an automated system (Bactec 9240, Becton Dickenson Microbiology system).
Case: A 75-year old man with ESRD was converted to CAPD in May 2004. After 2 months he developed cloudy PD effluent and abdominal pain but no fever. He was started on intraperitoneal (IP) vancomycin and oral (PO) levofloxacin according to the unit's protocol. Microscopic examination of the PD fluid revealed 333 neutrophils, 207 mononuclear cells. It signalled positive on day 5 of incubation but no organism could be seen on microscopy. After 4 days of incubation of the subculture plates, fine growth became visible. Microscopy of the growth showed a faint Gram-positive bacillus which was subsequently found resistant to all routinely tested antibiotics. Identification of the organism proved difficult by routine laboratory methods and was later identified as *M.chelonae* by the reference laboratory (Health Protection Agency, London). Antibiotic treatment was changed to PO clarithromycin and IP amikacin, the latter was subsequently changed to PO ciprofloxacin on the basis of sensitivity result. Clinical and laboratory signs of peritonitis persisted in spite of 2 weeks of antibiotics. The CAPD catheter was then removed which resulted in gradual resolution of symptoms. The catheter tip grew *M.chelonae*. Antibiotics were continued for 5 months. At one year of follow up the patient remains well and is free of any peritonitis related complications.
Conclusions: Atypical mycobacteria including *M.chelonae* should be considered in culture negative CAPD peritonitis. Investigation of CAPD peritonitis should include procedures for mycobacterial isolation. Removal of peritoneal catheter in addition to antibiotics was required in management of peritonitis.

P1136

Nontuberculous mycobacterial keratitis after photorefractive keratectomy for myopia

D. Mylona-Petropoulou, G. Vrioni, G. Ganteris, D. Miltsakakis, A. Kyratsa, C. Mougnai, A. Kouris, S. Levidiotou, H. Malamou-Lada *(Athens, Ioannina, GR)*

Objectives: To report a case of nontuberculous mycobacterial keratitis after photorefractive keratectomy for myopia.
Methods: A 45-year-old woman in good health developed increased conjunctival redness, swelling and marked decrease of vision in the right eye, 6 weeks after unilateral photorefractive keratectomy for myopia. Initial treatment included topical steroids, ofloxacin ophthalmic drops, and systemic treatment with fluconazole and vibramycin. After 4 more weeks without improvement, the patient was referred to our hospital. A diagnosis of infectious keratitis with corneal ulcer in the right eye was made. Corneal scrapings were sent for culture and microscopic examination, and therapy with vancomycin and tobramycin was initiated.
Results: Acid-fast bacteria were seen on smears. Cultures on Lowenstein-Jensen medium grew Mycobacterium chelonae complex (group III, M. abscessus) that was identified using two different reverse hybridization line probe assays: GenoType Mycobacteria CM (Hain Lifescience, Germany) and INNO-LiPA Mycobacteria v2 (Innogenetics, Belgium). Susceptibility testing was performed by E test method (Solna, Sweden) and the isolate was susceptible to amikacin and clarithromycin. Based on microbiological results, topical therapy using amikacin was initiated. After 5 weeks of treatment due to no-improvement, topical imipenem was added to her treatment regimen. All medications were discontinued 4 weeks later and final visual acuity was 7/10.
Conclusions: This case emphasizes the possibility of nontuberculous mycobacterial keratitis as a potential sight-threatening complication after photorefractive keratectomy for myopia and the possible difficulties in treating such infection. Early and accurate diagnosis can lead to prompt management of the infection with immediate and aggressive antibiotic therapy.

P1137

Leprosy elimination campaign in the Qazvin province, Iran, 2004

R. Qassemi Barqi *(Qazvin, IR)*

Introduction: Leprosy Elimination Campaigns are the whole innovative actions that, they are accomplished for leprosy cases finding particularly Multi Bacillary type of them. To promote the health staff's capacity, increasing of people's knowledge and participation of them about different activities for leprosy elimination, leprosy cases finding and treatment of them by Multi Drug Therapy are the main aims of the LEC. Most endemic countries have achieved LEC, from 1995.
Objective: The purpose of this study is leprosy new cases finding among house hold contacts of Previous patients with leprosy and also among natives of the endemic areas in the Qazvin province.
Materials and Methods: In this intervention and cross-sectional study, the first; names and addresses of the patients with leprosy that had been recorded & treated during 1958–2003 in the Qazvin province; are listed based on Qazvin Health Centre of province of documents. Then, household contacts of these patients were recognized and recorded locally. They were examined from having leprosy signs point of view by expert team. Cutaneous smears were prepared from suspected cases. Smears were studied for leprosy bacteriology by microscope.
Results: During study period 1768 household contacts of 319 previous patients with leprosy and 60 > 8 person from the endemic areas were examined. Suspect cases of leprosy were 258, that 13 cases of them were confirmed as new patients with leprosy (Incidence Rate = 1.7 /1000). From typing point of view 7 cases (54%) were Multi Bacillary, and 6 cases (46%) were Puci Bacillary. No one of the new patients were in < 20 years- old age group.
Discussion & Conclusion: The performance of LEC in the Qazvin province indicated action programme for the

Elimination of Leprosy in this province has achieved its goals in all districts (< 1/10000 population). Also, there was no one patient in < 20 years- old age group, that indicates success of the leprosy treatment by MDT in the past two-decade.

P1138

The first reported European case of intravascular catheter infection with *Mycobacterium neoaurum*

S. Musaad, N. Young, G. Robinson, D. Gascoyne-Binzi, A. Rajgopal, A. Barrett, D. Norfolk, T.A. Collyns *(Leeds, Newcastle, UK)*

Case Report: An 18 year old, newly diagnosed acute myeloid leukaemia (AML) patient had a Hickman line inserted prior to commencement of appropriate chemotherapy. He developed a pyrexia on day 2 post commencement of his second cycle of chemotherapy and blood cultures (BCs) through both lumina of his Hickman line yielded ''diphtheroid-like'' Gram positive bacilli (GPB). The organism was susceptible *in vitro* to vancomycin and a course of this agent resulted in clinical resolution. After his second cycle of chemotherapy was completed, the fever recurred, with no apparent focus except a tender line exit site. Line and peripheral BCs grew GPB as previously and further vancomycin therapy produced a good clinical response. Two days after starting the third cycle of chemotherapy, he became pyrexial with GPBs again recovered from Hickman and peripheral BCs. However, on light microscopy the organism appeared beaded and was acid and alcohol fast on Ziehl-Neelsen staining. The isolate required 48 hours incubation to produce visible colonies, which were pigmented yellow. It was identified definitively on the basis of the nucleic acid sequence of its I6SrDNA gene. This demonstrated 100% similarity with that of Mycobacterium neoaurum (strain 44074 DSM) as described in the RIDOM database (http://www.ridom-rdna.de). Interestingly, the organism was not recovered from mycobacterial BCs (Bactec 9050; Becton Dickinson). The mycobacterium was shown to be susceptible *in vitro* to rifampicin, ciprofloxacin and clarithromycin among other agents. The line was removed and the patient received 6 weeks of oral clarithromycin, ciprofloxacin and rifampicin. Routine and mycobacterial BCs were negative post line removal. He responded well to this treatment and remains in remission from his AML. *M. neoaurum*, a rapid growing chromogenic mycobacterium first described in 1972 from soil in Japan, has been reported from a sparse number of clinical cases world wide. This is the first reported case of Mycobacterium neoaurum line infection in Europe (Medline/PubMed). In accordance with previous published experience, the patient responded well to line removal and appropriate antimicrobial chemotherapy. This case also highlights the need for continual vigilance in the detection of emerging opportunistic pathogens in immunocompromised patients.

P1139

Impact of differents antimycobacterial drugs on the survival of patients with *Mycobacterium xenopi* pulmonary infection: 89 cases

C. Andrejak, F.X. Lescure, Y. Yazdanpanah, Y. Douadi, T. May, B. Hoen, P. Chavanet, G. Laurans, V. Jounieaux, J.L. Schmit *(Amiens, Tourcoing, Nancy, Besancon, Dijon, FR)*

Background: Only small series are available about *Mycobacterium xenopi* (MX) pulmonary infection (PI) without a real evaluation of the management. Impact of clarithromycin, rifamycins and ethambutol on the survival are discussed in the literature.

Methods: We reported a retrospective survival analysis of 89 consecutive patients (pts) with MX PI. The aim was to evaluate the interest of principal drugs used in the treatment of MX PI. The type of management and survival were recorded for each patient. Only ATS criteria pts were reviewed in 12 medical centres of North-East France between 1985 and 2003.

Results: Sex-ratio M/F was 66/23. Mean age was 49 ± 16. The mean duration of treatment was 7 ± 5 months with often a triple anti mycobacterial therapy. In first line, rifamycins (RFM) were used in 41 pts (46%), ethambutol (EMB) 36 pts (40%), clarithomycin (CLA) in 15 pts (17%). Only 33% of pts were alive after 36 months. 20 pts (23%) died before the end of treatment. Death was attributed to MX in 58%. Relapse was documented in 13 pts. Kaplan Meier survival analysis found variables significantly associated with a best prognosis: absence of neoplasm ($p = 0.002$), treatment vs abstention ($p = 0.007$), RFM and/or EMB containing regimen ($p = 0.02$). No significative difference was noted for CLA containing regimen. Cox multivariate analysis showed that treatment containing RFM and/or EMB was the only one significant independent positive prognostic factor ($p = 0.01$). Neoplasm ($p = 0.06$) was an independent trend.

Conclusion: The preliminary results of this study, the largest to our knowledge, showed the crucial role of RFM and/or EMB containing regimen in the treatment of pts infected with MX and the lack of impact of CLA on survival.

P1140

BCG infection following BCG therapy for bladder carcinoma: results of a multicentre study, about 23 cases

A. Godel, E. Bonnet, L. Porte, F. Busato, M. Alvarez, B. Doudie, P. Brouqui, H. Laurichesse, P. Massip, B. Marchou and the InfectioSud Group

Objectives: To describe epidemiological and clinical features of infections due to Mycobacterium bovis after BCG therapy for bladder carcinoma. To collect data on treatment options and evolution (with or without treatment).

Methods: We conducted a retrospective-prospective, descriptive, multicentre study including patients whose diagnosis of BCG infection following BCG therapy for urothelial carcinoma was assessed in Infectious Diseases Units in various University Hospitals in Southern France from January 2000.

Results: 23 cases were recorded. All patients were male, aged from 49 to 76 years (average = 63.2). Patients received an average of 4.5 BCG administrations before first clinical signs occurred. A traumatism during administration was noticed in 6 cases (26%). Twenty patients (83%) had an early disease. All the patients but one had fever. There was a systemic infection with general signs or located signs distant to the urinary tract in 22 cases (96%). Eight patients had respiratory signs (35%). Diagnosis was assessed by clinical history and negative search for ''usual'' infections (including common bacterial infections of the urinary tract or the prostate), and positive cultures for *M. bovis* [4 cases: positive blood cultures on specific media for mycobacteria (2),urine cultures (2)], and/or histologic findings compatible with a mycobacterial infection [liver granulomatosis (4), bone marrow granulomatosis (3), bladder granulomatosis (1), brain granulomatosis (1)], and/or abnormal images on CT-scan compatible with mycobacterial lesions. Among the 4 patients for which a medullogram was performed, 3 had signs of macrophagic activation. Inflammatory biological parameters

(ESR and/or CRP) were high in 15 patients (65%). Liver enzymes were increased in 16 patients (70%). Concerning treatment, three patients were not treated. Twenty patients (91%) received Rifampin and INH of which 11 (48%) received also Ethambutol. Eleven patients (48%) were also treated with corticosteroids. The outcome of the infection was favourable in all cases but one (who stopped his treatment after the first month).
Conclusion: BCG infection may occur many months after the end of the BCG therapy. The spectrum of clinical manifestations is very wide. Cultures for *M. bovis* are rarely positive and the diagnosis is suspected on clinical history, histological and tomodensitometric findings. Guidelines for treatment are needed.

P1141

Extrapulmonary tuberculosis in immunocompetent adults

T. Turunc, Y.Z. Demiroglu, H. Uncu, S. Colakoglu, H. Arslan *(Ankara, TR)*

Objectives: Although tuberculosis usually attacks the lungs, resulting in the pulmonary tuberculosis form, other organs may also be affected, leading to extrapulmonary tuberculosis (EPT) or disseminated tuberculosis. This study retrospectively analysed the incidence, clinical sites and risk factors for EPT in 46 patients who admitted to the hospital with EPT between 1 January 2003–1 January 2005 in Turkey where tuberculosis is still frequently seen.
Methods: A total of 46 patients who were diagnosed as EPT between January 2003 and January 2005 were enrolled in this retrospective study.
Results: Forty six cases were diagnosed as EPT during the 2 year period. Twenty two were males (47.8%) and 24 females (52.2%). The mean age of the patients was 50.6 years (range, 17–80 years). Of the 46 patients, tuberculous lymphadenitis was diagnosid in 18 (39.1%), genitourinary system tuberculous in 8 (17.3%), Pott's disease in 7 (15.2%), miliary tuberculosis in 3 (6.5%), peritonitis tuberculous in 1 (2.1%), skin tuberculosis in 1 (2.1%), bone tuberculosis in 1 (2.1%), and joint tuberculosis in 1 (2.1%). Symptoms were usually non-specific, and related to the site of infection. The most common symptoms were fever (20/46, 43.4%), fatigue (15/46, 32.6%). Concurrent disease, previous history of tuberculosis and a history of contact with an infected patient were determined to be the major risk factors for EPT. Of all patients, 11 (23.9%) had a previous history of pulmonary tuberculosis, 8 had chronic renal failure (17.3%), and diabetes mellitüs 5 (10.8%). EPT was diagnosed by histopathologic methods in 27 (57.8%) and by microbiologic methods in 13 (28.2%) of the patients. In six of the cases, and cultures grew no bacteria EPT was diagnosed by the helps of clinical, laboratory, imaging, and/or histopathological studies. Surgery was required in 5 patients; 2 had tuberculous lymphadenitis, 2 had Pott's disease, and 1 had tuberculous osteomyelitis. In our study, 3 of the patients (6.5%) died one had miliary tuberculosis, and 2 patients had tuberculous meningitis.
Conclusion: Physicians should be aware of unusual presentations and localizations of tuberculosis and should consider EPT in the differential diagnosis of any patient with non-specific symptoms such as fever, fatigue, headache, night sweat, low-back pain, and weight loss.

P1142

Cigarette smoking and its association with pulmonary tuberculosis in adults

M. Rasoolinejad, A. Farazi, M. Haj Ab-dul Baghi, P. Hashemi, P. Kheirandish *(Tehran, IR)*

Objectives: Smoking and tuberculosis have been widespread for many years in the world, and are the two health problems of developing countries.Smoking and tuberculosis are more prevalent in men than women. The aim of this hospital based case-control study is to determine the effect of smoking on pulmonary TB in male adults.
Methods: The subjects were 77 male (age between 21–50 years) with pulmonary TB. The controls were 154male patients matched for age and socio-economic conditions chosen from non-TB patients admitted to hospital from June 2003 to August 2004.In order to reduce bias, the subjects were selected from different wards from the patients without HIV or diabetes mellitus based on inclusion and exclusion criteria. The data were collected by direct interview using Questionnaires, and were analysed using odd ratio Chi-square, mantel-haenzell and conditional Logistic regression.
Results: Based on the univariate analysis, cigarette smoking at the time of study (OR = 2.281, P = 0.004), the age at which smoking started (OR=2.951,P = 0.01 to OR = 3.463, P=0.001), duration of smoking (P = 0.043,OR = 2.32 to OR = 2.361, P = 0.018) and the number of cigarettes smoked per day (OR = 0.632, P = 0.003 TO OR = 5.397, P = 0.001) were significantly associated with pulmonary tuberculosis. Multivariate conditional logistic regression analysis showed that smoking as an independent risk factor increased the risk of pulmonary tuberculosis (P = 2.172, P = 0.009). In this analysis the age at which smoking started (OR = 9.296, P = 0.001 to OR=14.32, P = 0.0001) and the number of cigarettes smoked per day (OR = 5.15, P = 0.008 to OR = 8.54, P = 0.031) significantly increased the risk of pulmonary tuberculosis.
Conclusion: Based on the results, smoking is an independent risk factor for pulmonary tuberculosis; there is a definite association between these two matters. Therefore an effective antismoking campaign is expected to have positive repercussion on TB prevalence, and smoking cessation must be considered and promoted by all levels of health care providers.

Experimental infections: treatment and pathogenesis

P1143

Does reduced susceptibility to disinfectants compromise colonisation and persistence of *Salmonella enterica* serovar Typhimurium in chickens?

S.W. Cooles, M.A. Webber, L.P. Randall, N.G. Coldham, L.J.V. Piddock, M.J. Woodward *(Addlestone, Birmingham, UK)*

Objectives: Reduced susceptibility to some disinfectants has been linked with reduced susceptibility to some antibiotics. The use of disinfectants may select for mutants with reduced susceptibility to both disinfectants and antibiotics, and it would be of concern if such mutants persist and accumulate within the food chain. In this study the ability of mutants derived from *Salmonella* Typhimurium following exposure to disinfectants to colonise and persist in chickens was compared to that of their isogenic parents in the day-old chick model. Mutants arising from exposure to a common domestic chlorophenol disinfectant (Triclosan) and an aldehyde-based farm disinfectant (Superkill) were studied.
Methods: Minimum Inhibitory Concentrations (MIC) of various disinfectants and antibiotics were determined by the agar

dilution method. Mutants of *Salmonella* Typhimurium were selected by plating up to 10^{10} colony forming units (cfu) on media supplemented with 1 – 2x the MIC of disinfectant. Three mutants exhibiting low (4 mg/L), medium (32 mg/L) and high (>128 mg/L) level resistance to triclosan and two exhibiting increased tolerance to superkill were selected. 1-day-old chicks were infected by oral gavage (c. 10^4 cfu per bird, c. 1:1 mix of mutant and parent strain) in competitive index experiments. Infection was monitored by twice weekly cloacal swabbing for ≥ 27 days post-infection. Weighed swabs were vortexed in saline (1 mL), diluted and plated (0.1 mL) onto media with or without antibiotic to differentiate between strains. Colonies were counted after overnight incubation at 37°C. Infection was calculated as cfu/g of faecal matter.

Results: All mutants showed reduced susceptibility to some clinical antibiotics. The mutant strains were significantly less able to persist than their parent strains, but persisted in the chicken gut and in some cases, even 23 days post-infection, they amounted to approximately 1% of the total *Salmonella* population of 10^6 cfu/g faecal matter.

Conclusion: Exposure of *Salmonella* Typhimurium to disinfectants gave rise to mutants with reduced susceptibility to disinfectants and/or antibiotics. Compared to their isogenic parent strains, mutants were still able to persist in the chicken gut to the end of the experiment.

P1144

A study on rat's pulmonary inflammatory reaction induced by N-protein of SARS-CoV and effects of glucocorticoids on it

L. He, D. Hao, J. Qu *(Shanghai, CN)*

Objective: To study rat's pulmonary inflammatory reaction induced by N-protein of SARS-CoV and effects of glucocorticoids.

Methods: Rat's pulmonary inflammatory reaction were induced by intratracheally installation of N-protein of SARS-CoV with a dose of 0.2 mg/kg. Rats were randomly divided into four groups: saline control group (Nc), N-protein group one (P1, 6 h), N-protein group two (P2, 24 h), N-protein+dexamethasone group (D, 10 mg/kg). Their blood, bronchial alveolar lavage fluid(BALF) and lung tissue were collected after challenging. Cytological and histopathologic changes of lung tissue were observed. The wet/dry ratio (W/D) of lung tissue were determined. Interleukin-6(IL-6), interleukin-10(IL-10) and transforming growth factor-B1 (TGF-B1) of serum and BALF were measured by ELISA.

Results: (1) Compared with Nc group (68.42 ± 13.07)%, lymphocyte percentage of peripheral blood of P2 group (50.5 ± 14.36)% decreased significantly ($P < 0.05$); Compared with Nc group ($P < 0.01$) and P2 group ($P < 0.01$), lumphocyte percentage of peripheral blood of D group decreased significantly; Compared with Nc group [(5.86 ± 2.25^) x 10^9] and P2 group [(4.83 ± 1.49) x 10^9], WBC total number of peripheral blood of D group [(1.96 ± 1.30) x 109] decreased obviously ($P < 0.01$). (2) Compared with Nc group [(95 ± 29) x10^7], WBC total number of BALF of P2 group [(160 ± 60) x 10^7] increased significantly ($P < 0.05$); WBC total number of BALF of D group [(62 ± 23) x 10^7] decreased obviously compared with P2 group; Analysis of differential cell counts in BALF indicated that the majority of infiltrating cells were alveolar macrophage in every group. (3) After N-protein challenging, W/D ratio were significantly high in both P1 group and P2 group than Nc group [(5.18 ± 0.29), (5.19 ± 0.34) vs (4.77 ± 0.27), $P < 0.05$], W/D ratio in D group (4.70 ± 0.18) decreased noticeably compared with P2 group ($P < 0.01$). (4) Compared with Nc group, IL-6, IL-10, TGF-B1 levels in both serum and BALF of P1 group increased significantly ($P<0.01$), these cytokines levels in both serum and BALF of P2 group were higher than P1 group ($P < 0.01$); these cytokines levels in D group decreased noticeably compared with P2 group ($P < 0.01$).

Conclusions: N-protein of SARS-CoV can lead to rat's pulmonary inflammatory reaction / acute lung injury which related to the rise and unbalance of pro-inflammatory and anti-inflammatory cytokines; Glucocorticoids can effectively alleviate pulmonary inflammatory reaction induced by N-protein of SARS-CoV.

P1145

Brain damage in pneumococcal meningitis: establishment of an infant mouse model

D. Grandgirard, O. Steiner, S.L. Leib *(Bern, CH)*

Objective: To establish a model of pneumococcal meningitis in infant mice, that reproduces the brain injury pattern found in the human disease.

Background: Brain damage, specifically neuronal necrosis in the cerebral cortex and apoptosis in the dentate gyrus of the hippocampus contribute to the high incidence of neurological sequelae occurring in up to half of children that survive pneumococcal meningitis.

Methods: Eleven days old mice from defined strains (C57BL/6 n = 136, CD-1 n = 42, BALB/c n = 14) were infected by intracisternal injection of 10 microL of saline containing log10 5.2 ± 0.5 colony forming units/ml of a clinical isolate of Streptococcus pneumoniae serotype 3. At 16 h after infection, cerebrospinal fluid was sampled to document pneumococcal meningitis and antibiotic therapy (ceftriaxone; 100 mg/kg; s.c.; bid) was initiated. Endpoints assessed included mortality within 40 h after infection and the occurrence of cortical and hippocampal brain damage by histomorphometry.

Results: Mortality was lowest in C57BL/6 mice, but did not correlate with the size of the inoculum used for infection. Histological evaluations of brain sections showed occurrence of neuronal apoptosis in the dentate gyrus in 38% of C57BL/6 and 10% of CD-1 mice. Necrotic damage in the cortex was exclusively observed in C57BL/6 mice. The findings are summarized in the table below:

Mouse strain	**Mortality** (%) [Spontaneous death/euthanized due to terminal illness within 40 h after infection]	**Hippocampal Apoptosis** (%) [animals with an apoptosis score > 0.25]	**Cortical Necrosis** (%) [animals showing cortical damage]
C57BL/6	48 (35)	33 (38)	13 (15)
CD1	21 (50)	2 (10)	0 (0)
BALB/c	14 (100)	0 (0)	0 (0)

Conclusions: The newly established infant mouse model of pneumococcal meningitis is unique, in that it exhibits neuronal apoptosis in the hippocampus and neuronal necrosis in the cerebral cortex. This characteristic injury pattern causes this model to be particularly useful as it mimics the histomorphological findings in children suffering from the disease. Thus, this model will allow the use of transgene technology as a new tool to investigate how bacterial meningitis causes brain damage in children.

P1146

PCR and culture correlate well for measuring cure rates for short course treatment of infections with the Lyme disease spirochete

C. Pavia, D. Liveris, S. Bittker *(Old Westbury, Valhalla, US)*

Objectives: To compare the sensitivity of PCR with standard culture techniques, using BSK media, for measuring the curative effects of short course treatment with the antibiotic ceftriaxone (CTX) against Borrelia burgdorferi (Bb) infection in mice.
Methods: Tissue samples were subjected to analysis by polymerase chain reaction (PCR), following our previously published procedures. DNA amplification was performed using FS1 and P19 as probes for the JS1-JS2 primers and the IS1-IS2 amplicons. For the mouse infectivity studies, we used 2 different North American Bb isolates, known as strain 297 and strain BL206 which we have used in previously published studies. Separate groups of C3H mice were infected intradermally with 100,000 culture-grown, low passage Bb of either strain, having an mbc for CTX ranging from 0.025–0.050 μg/ml. Two weeks later, the mice were given a single intramuscular dose of saline or CTX (50 mg/kg). One week after treatment, cultures of the urinary bladder and the ears were established in BSK media, or were processed separately for PCR analysis. Aliquots of each extract culture were examined, at weekly intervals, for the presence of spirochetes using dark field or phase-contrast microscopy.
Results: In infected but untreated control mice, live Bb were readily culturable from extracts of the urinary bladders and ears. In contrast, it was found that the one-day, single-dose regimen of CTX was 100% effective in sterilizing the 2 selected tissue samples from mice infected with either strain of Bb. It was also found that, of the culture-positive specimens, 100% yielded a positive signal for the presence of Bb. For the culture-negative tissue samples, 80% were negative by PCR.
Conclusions: These experiments show that short course treatment with CTX is effective against experimental Bb infection, based on combined PCR analysis and culture techniques. Given the complexity and time required for culture, PCR is a useful technique for verifying the effectiveness of antibiotic treatment regimens against Lyme disease in various experimental animal-model systems.

P1147

Efficacy of aminocandin (IP960/HMR3270) in temporarily and persistently neutropenic murine models of disseminated aspergillosis

P. Warn, A. Sharp, D.W. Denning *(Manchester, UK)*

Background: Aminocandin (AC) (IP960) is an echinocandin antifungal agents with broad spectrum antifungal activity. Patients with invasive aspergillosis often have compromised immune systems. Concerns have been raised that due to the mode of action of the candins an effective immune system is required to clear damaged hyphae. We compared the activity of aminocandin® (AC), micafungin® (MFG) and caspofungin® (CAS) in persistently neutropenic (PN) mice; AC, itraconazole (ITZ) and amphotericin B (AMB) in temporarily neutropenic (TN) mouse models of disseminated aspergillosis.
Method: Male CD1 mice were infected with either *A. fumigatus* AF293 (TN), AF91 (ITZ resistant) (TN) or CBS144 (PN). Mice were compromised using either one or multiple doses of 200 mg/kg cyclophosphamide every 3 days starting day -3. Mice were treated IV 4–24 hours later with either 10, 4 or 2 mg/kg/day or 4 mg/kg every 2 days AC, MFG or CAS (PN) or 0.25–5 mg/kg AC, 25 mg/kg ITZ, 5 mg/kg AMB (TN). Pk samples (0.25–2 mg/kg) were taken from a 2nd set of infected mice 4 days post infection. One set of mice were killed 96 hours post infection, a 2nd set were treated for 10 days then 4–6 days observation before assessment of tissue burden.
Results: Mortality in untreated mice was 80–100%. Tissue burdens 4 days post infection in control mice were high with the liver (log 3.8 cfu/gm), spleen (log 3.7 cfu/gm) and kidneys (log 4.2 cfu/gm), the main target organs. Treatment reduced burdens in all organs in PN mice particularly the kidneys which had a log 1.5 cfu/gm reduction. Survival was excellent in both PN and TN models after treatment. In the PN model survival was AC 96%, MFG 93% and CAS 96%. In the TN models survival was 5 mg/kg AC 100%, 1 mg/kg AC 90%, 0.25 mg/kg AC 20%, ITC (AF293 only) 70%, AMB 90%. Mean burdens in the PN model at the end of the observation period were low at log0.5, log0.3 and log1.95 cfu/gm in the liver, spleen and kidneys respectively. Total clearance of Aspergillus in the PN model was found in 7/28, 4/27 and 8/28 survivors treated with AC, MFG and CAS respectively. PK of AC demonstrated peak and AUC increased in a linear fashion by dose, with AUC and half-life of 16–176 mg-hr*hr/L and 27–45 hrs for 0.25–2 mg/kg/day.
Conclusions: AC at doses in excess of 1 mg/kg/day were highly effective both at improving survival and reducing tissue burdens even in persistently neutropenic mice with disseminated aspergillosis. Alternate day dosing of AC at twice the daily dose was also very effective.

P1148

Therapeutic drug monitoring of 5-flucytosine in children aged under 12 years. An 11-year review of serum concentrations from a United Kingdom clinical assay reference laboratory

M. Soltani, C.M. Tobin, K.E. Bowker, A.M. Lovering, A.P. MacGowan *(Bristol, UK)*

Objectives: 5-Flucytosine is an antifungal drug, used for the treatment of serious infections caused by Candida or Cryptococcus species. In the UK, the pre and post dose therapeutic ranges are 30–40 mg/L and 70–80 mg/L, respectively. Drug monitoring is recommended in children, who have unpredictable pharmacokinetics, and in patients with an impaired renal function, to prevent bone marrow toxicity, which may occur due to drug accumulation.
Methods: 5-Flucytosine was assayed by high performance liquid chromatography (HPLC). Patient data were collected retrospectively from the hospital laboratory information management system.
Results: From February 1994 to February 2005, pre and post dose samples were received from more than 100 neonates and children, aged up to 12 years, from 90 UK hospitals. For patients aged between 1–30 days (n = 93), 31–90 days (n = 68), and 91 days –12 years (n = 63), 61.3%, 42.7% and 22.4% respectively of pre dose samples were above the recommended range of 40 mg/L. The mean values of pre dose concentration of each group were 51.7 mg/L, 46.1 mg/L and 31.2 mg/L respectively. For post dose concentrations 35%, 32% and 19.3% were above the recommended range of 80 mg/L. Conversely, 20.4%, 36.8% and 56.7% of pre dose samples and 46.3%, 62% and 63.2% respectively of post dose samples were lower than the recommended range. Only 18.3%, 20.7% and 20.9% of pre dose samples and 18.8%, 10% and 7% respectively, of post dose samples were within normal ranges for the three age groups.

ISSN: 1470-9465

Conclusion: An appreciable number of pre and post dose 5- Flucytosine concentrations in the three groups of children were outside the recommended ranges. Serum concentrations were significantly higher in the youngest age groups, suggesting that the standard dose of 200 mg/kg may not be appropriate in neonates. Nevertheless our results show that it is important to monitor serum levels during therapy in all children.

P1149

Experimental dermatophytosis in guinea pigs: is voriconazole an option for treatment?

D.M. Saunte, N. Frimodt-Moller, E.L. Svejgaard, M. Haedersdal, M.C. Arendrup *(Copenhagen, DK)*

Objectives: Standard treatment of tinea capitis caused by Microsporum (M.) species has for many years been oral griseofulvin which, however, is no longer marketed in all countries. Voriconazole (VCZ) has been demonstrated to inhibit *M. canis in vitro*. The aim of this study was to investigate the efficacy of voriconazole in a guinea pig model for dermatophytosis.
Methods: Sixteen female Harlan (HsdSsc:AL) guinea pigs (n = 8/group) were inoculated with 5×10^5 *M. canis* cells/ml on razed skin. The VCZ-group was dosed p.o. 20 mg VCZ/kg/day in 12 days (day 3–15). The control group was left untreated. The guinea pigs were evaluated clinically twice a week and mycologically once a week. The clinical evaluation consisted of a redness score (0 = normal, 1 = pink, 2 = red, and 3 = violet) and a lesion score (normal = 0, papule = 1, scales = 2, scales (thin) and ulcers = 3, and scales (thick) and ulcers = 4). The mycological examination consisted of direct microscopy and culture of skin scrapings using Sabouraud-glucose-agar with chloramphenicol & cycloheximid incubated at 25ordm; C for four weeks. Species identification was performed based on micro- and macromorphology and the number of colonies was noted.
Results: All animals had proven mycological infection. The VZC-treated group had significantly lower redness and lesion scores as compared to the control group (P = 0.03 (Mann–Whitney)). After 12 days of treatment 8/8 in the VCZ-treated group was microscopy negative and 7/8 culture negative in contrast to the control group where 8/8 animals were microscopy and culture positive and with significantly higher colony counts (mean colony count: <1 versus 28 in the control group, P = 0.0002).
Conclusion: These data show that oral VCZ treatment is efficacious for dermatophytosis caused by *M. canis* in an animal model and therefore may be a future alternative for tinea capitis in humans especially when griseofulvin is not available.

P1150

Moxifloxacin is markedly more effective than levofloxacin, gatifloxacin or azithromycin in the treatment of pneumococcal pneumonia in mice caused by a macrolide-resistant isolate

D.J. Bast, L. Dresser, C.L. Duncan, L.A. Mandell, D.E. Low, J.C.S. de Azavedo *(Toronto, CA)*

Objective: Macrolides are commonly used in the empiric treatment of community-acquired pneumonia (CAP). However the increased worldwide prevalence of macrolide resistance among Streptococcus pneumoniae (SPN), the most common cause of CAP, has promoted the use of other antibiotic classes like the fluoroquinolones (FQ). We tested the efficacy of 3 FQ (moxifloxacin [MXF], gatifloxacin [GFX], & levofloxacin [LFX]) & azithromycin (AZI) in the treatment of pneumococcal pneumonia caused by a macrolide-resistant isolate.
Methods: Pneumonia was induced in Swiss Webster mice by endotracheal inoculation with a macrolide-resistant (AZI MIC 256 μg/ml; ERY MIC 1024 μg/ml) but FQ susceptible (MXF MIC 0.06 μg/ml; GFX MIC 0.12 μg/ml; LFX 0.5 μg/ml) serotype 3 SPN strain. In a bid to produce levels of drug exposure in mice approximating that in humans, single-dose pharmacokinetics were evaluated for each drug (MXF [50 mg/kg]; GFX [50 mg/kg]; LFX [10 mg/kg]; AZI [40 mg/kg]). Animals received antibiotics subcutaneously at 8-(MXF, LFX) and 12-h intervals (GFX), or orally at 24-h intervals (AZI), with antibiotic treatment beginning at 8-h post-infection and lasting for up to 4 days. Temperatures of all mice were measured prior to, and during treatment to assess the severity of infection and determine clinical improvement or failure. Only mice regarded as moderately ill (< 32 but > 30 degrees C) were used in this study. A temperature of < or equal to 30 degrees C was used as the endpoint since previous studies have shown death to be imminent at this cut-off. Rates of survival, lung eradication & resistance selection were determined & compared among all drugs.
Results: Administration of a FQ, and in particular MXF (at free-drug AUC [0–24 h]: MIC ratios of greater than 40) produced superior rates of survival & bacterial clearance compared with AZI (MXF, 142; LFX, 48.6; GFX, 223). MXF protected all 25 mice (100%) compared with GFX (19/23 [83%]), LFX (19/24 [79%]) & AZI (9/34 [27%]) (P < 0.05). Furthermore, all mice treated with MXF or GFX showed complete eradication (100%) from the lungs compared with LFX (83%) & AZI (24%). No changes in susceptibility were observed for any of the isolates recovered.
Conclusions: The FQ, & in particular MXF, are highly effective in the treatment of pneumococcal pneumonia caused by a macrolide resistant isolate.

P1151

Moxifloxacin is highly active in a murine Gelfoam® implant model of complicated skin and skin structure infections

K. Merfort, S. Obertegger, R. Endermann *(Wuppertal, DE)*

Objectives: MXF has recently been indicated for the treatment of complicated skin and skin structure infections (cSSSIs), so this study was designed to evaluate the efficacy of moxifloxacin (MXF) vs methicillin-susceptible Staphylococcus aureus (MSSA) in a murine model of cSSSI and to compare MXF with levofloxacin (LFX), vancomycin (VAN), linezolid (LIN), amoxicillin-clavulanate (AMOX-CLAV) and ceftriaxone (CEF).
Methods: Gelfoam® pieces (1 x 1 cm) were implanted subcutaneously on the back of mice (5 mice/group). Abscess-like fibrotic capsules formed round the implants after 3 days. A suspension (50 μl) of S. aureus DSM 11823 was injected into the Gelfoam® – a technique known to produce implant colonization. Antibiotics at doses of 2.5 and 10 mg/kg were administered intravenously b.i.d. for 3 days, starting 2 h after implant infection. On day 3, implants were removed, homogenized and the viable bacterial load in the Gelfoam® homogenates determined by plating serial tenfold dilutions on sheep blood agar plates. Bacterial colony forming units (CFU) were counted after overnight incubation of the plates at 37°C.
Results: The results from studies on two different days with different inocula are shown in the table. At 10 mg/kg, MXF showed a more pronounced reduction in colony forming units (CFU) than LFX and VAN. No reduction in CFU was obtained for LIN and CEF at the same dose.

		Bacterial cell counts in the implant (CFU/mL); Mean of n=5		
		2.5 mg/kg	10 mg/kg	Control
Study 1	MXF	2.62×10^5	6.03×10^1	6.59×10^7
	LFX	3.29×10^7	4.29×10^3	6.59×10^7
	VAN	1.52×10^8	9.63×10^3	6.59×10^7
	LIN	2.83×10^7	2.98×10^7	6.59×10^7
Study 2	MXF	4.02×10^4	5.02×10^3	5.3×10^6
	AMOX-CLAV	1.61×10^4	7.3×10^2	5.3×10^6
	CEF	9.3×10^7	8.7×10^6	5.3×10^6

Conclusion: In the Gelfoam® implant model of cSSSI, eradication of MSSA with MXF is comparable to that with AMOX-CLAV and superior to LFX, VAN, LIN and CEF. Therefore, MXF is likely to be an effective treatment for abscesses caused by MSSA.

P1152

Efficacy of continuous or bolus IV ceftazidime and combination therapy in septic neutropenic mice

J. Buijs, A.S.M. Dofferhoff, J.W. Mouton, J.W.M. van der Meer *(Heerlen, Nijmegen, NL)*

Low dose ceftazidime inhibits Penicillin-Binding Protein (PBP)-3 in Gram-negative bacteria, causing filament-formation, which is related to high endotoxin-releases *in vitro*, and possible high cytokine responses *in vivo*. High dose ceftazidime causes PBP-1 binding, inducing bacterial lysis. Earlier studies showed that continuous infusion of ceftazidime above 40 mg/kg prevents filament-formation, and leads to early high cytokine responses by a rapid lysis of bacteria. We hypothesized that, during filament-formation, endotoxin is retained in the bacterial cellwall, inducing lower initial cytokine releases. A consequence might be the risk of a postponed, but potentially more severe inflammatory response once bacteria are killed by further treatment.

Objective: To investigate bacterial killing and cytokine responses after exposure of bacterial filaments to a beta-lactam antibiotic with different PBP-affinity.

Methods: In 3 experiments, 54 neutropenic Swiss mice were challenged with 1.0×10^7 CFU of *E.coli* ATCC 25922 in thigh muscles. After inoculation, 80 mg/kg ceftazidime was given either as an IV bolus or continuous infusion (T = 0). 4 Hours after start of ceftazidime, mice were injected with meropenem, to enhance bacterial killing. On T = 4, 6 and T = 8 hours, mice were sacrificed for measurements of circulating IL-6, TNFalfa, bacterial counts and morphology.

Results: After 4 hours continuous ceftazidime bacteria showed rod-shapes and debris. Bolus-treated mice showed filaments IM. Higher IL-6 concentrations ($p = 0.004$) were found after 4 hours of continuous ceftazidime. After subsequent meropeneminjection, IL-6 and TNF rose to higher levels in the continuous group untill t = 6 hours ($p = 0.011$). On T = 8 hours cytokine concentrations are lower, but still above the level of bolus-treated mice ($p = 0.005$). CFUcounts were lower in continuous treated mice. Both results aim at a more effective bacterial lysis.

Conclusion: Continuous infusion of 80 mg/kg ceftazidime prevents filament-formation and leads to significant higher concentrations of circulating IL-6 and TNF-alfa in contrast to IV bolus. After addition of meropenem cytokines rise even further, reflecting more lysis and concomittant release of endotoxin in the initially continuously treated group. This is confirmed by bacterial counts. So filament-formation, as shown after bolus ceftazidime, is associated with a less efficacious antibiotic induced bacterial lysis, and might thus be disadvantageous.

P1153

Amoxicillin early and delayed treatment of serotype 6 pneumococcal acute otitis media in previously immunised vs. non-immunised gerbils

C. Martínez-Marín, G. del Prado, L. Huelves, M. Gracia, C. Ponte, C. Cenjor, M.J. Giménez, L. Aguilar, F. Soriano *(Madrid, ES)*

Objective: To study the effect of previous immunisation in the outcome of pneumococcal acute otitis media (AOM) treated with Amoxicillin (AX).

Methods: Two serotype 6B pneumococcal clinical isolates with AX MIC of 2 (strain A) and 8(strain B) mg/L were used. Animal were weekly intraperitoneally (i.p.) immunised with whole cell heat inactivated inoculum of each strain for 5 weeks. Immunised (IMM) and non-IMM animals (8 per group) were bilaterally inoculated into the middle ear bulla with 20 microL of broth containing approximately 106 cfu. Doses of AX (5 mg/kg) were subcutaneously given in an early (2, 4.5, and 7 h post-inoculation, p.i.) or delayed (18, 20.5, 23, 25.5, 28, and 30.5 h pi) schedule to previously IMM and non-IMM animals: Efficacy was evaluated by middle ear fluid colony counting and body weight, 48 h p.i.

Results: There were not differences in AX AOM treatment outcome between previously IMM and non-IMM animals. AX was not efficacious in AOM caused by the resistant strain (B). AX treatment was efficacious in AOM caused by the strain with AX MIC in the upper limit of susceptibility (A) only when the antibiotic was early administered.

Strain	Group	Positive ears (%)	Colony counting (log_{10} cfu)	Weight loss (%)
A	Non-IMM	75.0	1.8±1.1	11.2±2.8
	IMM	62.5	1.6±1.0	13.3±2.0
	Non-IMM + AX early	6.25	0.7±0.3	2.4±2.1
	IMM + AX early	0.0	<0.6	3.0±1.4
	Non-IMM + AX delayed	61.1	0.9±0.4	9.1±1.8
	IMM + AX delayed	62.5	1.3±0.9	12.6±1.5
B	Non-IMM	100	2.9±0.6	11.5±2.3
	IMM	93.7	2.8±0.9	11.4±2.7
	Non-IMM + AX early	93.7	2.1±0.8	9.8±2.2
	IMM + AX early	93.7	1.9±0.9	8.5±3.9
	Non-IMM + AX delayed	87.5	2.0±0.8	7.3±2.0
	IMM + AX delayed	81.2	2.3±1.2	10.1±3.2

Conclusions: In this model, previous serotype 6 pneumococcal immunisation did not influence outcome of AX treatment of AOM caused by AX sensitive or resistant strains.

P1154

Efficacy of amoxicillin-clavulanate in an experimental murine pneumonia model caused by AmpC non-hyperproducing clinical isolates of *E. coli* resistant to cefoxitin

F. Docobo-Pérez, F. Fernández-Cuenca, M.E. Pachón-Ibáñez, A. Pascual, C. Pichardo, L. Martínez-Martínez, J. Pachón *(Seville, ES)*

Objectives: The aims of the study were determine the *in vitro* activity of AMC and its efficacy in an experimental pneumonia

model caused by a new phenotype of AmpC non-hyproducing *E. coli* resistant to FOX.
Methods: *In vitro* studies: two clonal related strains (Rep-PCR) resistant to FOX were used. Ec985 (no TEM-1 producer) susceptible to AMX, AMC, CTX, and Ec571 (TEM-1 producer) susceptible to AMC and CTX. MIC and bactericidal activity (time-kill curves) were measured. PK and PD parameters (Cmax, t1/2, and time above MIC) were determined after a single dose of each antimicrobial. *In vivo* studies: 1st Phase. Characterize an experimental pneumonia model by *E. coli* in C57BL/6 mice (16–20 g), with a bacterial inoculum of 8–9 log10 cfu/ml. 2nd Phase. Animals were grouped in controls (CON) without treatment, AMX (50 mg/Kg) each 2 hours (5 doses), AMC (50/5 mg/Kg) each 2 hours (5 doses), and CTX (50 mg/Kg) each 3 hours (4 doses). Analysed variables: log10 cfu/g lung tissue. Statistical analysis: ANOVA, post-hoc Tukey, and Dunnett test.
Results: *In vitro*: MIC (mg/l): Ec985 AMX = 8, AMC = 8, CTX ≤ 0.5, FOX = 128; Ec571 AMX = 512, AMC = 8, CTX ≤ 0.5, FOX = 128. Bactericidal antibiotics: Ec985 AMX, AMC, and CTX (1xMIC, 2xMIC, 4xMIC, Cmax). Ec571 AMX, AMC, and CTX (1xMIC, 2xMIC, 4xMIC, Cmax). *In vivo*: PK/PD (Cmax [mg/l]; t1/2 [h]; time above MIC [h]): AMX (50 mg/kg) 15.1; 0.28; 0.48. AMC (50/5 mg/kg) 17.9; 0.4; 0.55. CTX (50 mg/kg) 57.6; 0.27; 1.89.AMX, AMC, and CTX reduced the bacterial lung concentration of Ec985 compared with CON ($p < 0.001$), no differences between the treatments were found. AMC and CTX reduced the bacterial lung concentration of Ec571 respect to CON ($p < 0.001$). No differences between the treatments were found.
Conclusions: AMC is effective in the treatment of the experimental murine model caused by this new phenotype of non AmpC hyperproducer *E. coli*, resistant to FOX.

P1155

Telithromycin is effective against susceptible, fluoroquinolone-resistant and macrolide-resistant strains of *Streptococcus pneumoniae* in a murine model of pneumonia

J.C.S. de Azavedo, C. Duncan, L. Dresser, L.A. Mandell, D.E. Low, D.J. Bast *(Toronto, Hamilton, CA)*

Objective: Resistance to commonly utilized antimicrobials such as the β-lactams, macrolides and fluoroquinolones (FQ) poses a challenge with respect to empiric treatment for community acquired pneumonia. Telithromycin (TEL), a ketolide, has high activity against resistant strains and has been approved for the treatment of respiratory tract infections. We undertook to examine its efficacy *in vivo*, in comparison with azithromycin (AZM), against a susceptible strain, a FQ-resistant strain (ciprofloxacin [CIP} MIC 4 mg/L) with a ParC mutation, and a macrolide resistant strain harbouring an Erm methylase.
Methods: A susceptible serotype 3 strain A66 (levofloxacin [LVX] MIC 0.5 mg/L; TEL MIC 0.004 mg/L; AZM MIC 0.125 mg/L), its isogenic derivative C98, with a Ser-79-Phe substitution in ParC (CIP MIC 4 mg/L, LVX MIC 2.0 mg/L, TEL MIC 0.004 mg/L; AZM MIC 0.125 mg/L) and a macrolide-resistant serotype 3 strain R326 (LVX MIC 0.5 mg/L; TEL MIC < 0.015 mg/L; AZM MIC > 256mg/L), were used to infect groups of 34 – 46 Swiss Webster mice endotracheally with 10E4 to 10E5 log-phase CFU. Single-dose pharmacokinetics were tested in mice in order to determine drug levels approximating human kinetics. TEL (24 mg/kg q12 h) and AZM (40 mg/kg q24 h) were administered orally 6 – 12 h post-infection and for up to 4 days. Surface temperature (ST) was used to assess disease severity & to stratify mice prior to treatment into moderately ill [MI]: > 32°C & severely ill [SI]: < 32°C but > 30°C. Mice were euthanized if their ST dropped to < 28°C. Bacterial counts from lungs of all mice were determined using an automated spiral plating system.
Results: TEL showed complete eradication in all mice infected with the susceptible and FQ-resistant strains with 95 – 100% survival rates among MI mice and 88 – 100% survival rates among SI mice. By comparison, AZM showed no eradication in 9% of mice infected with these strains despite survival rates of 100% among MI mice and 78 – 100% in SI mice. Predictably, in mice infected with the macrolide-resistant strain, AZM showed eradication and survival rates of only 22% and 25% respectively, whereas TEL showed eradication and survival rates of 85% and 88% respectively ($p < 0.01$).
Conclusions: TEL is highly effective against susceptible, FQ-resistant and macrolide resistant strains of SPN and provides a useful drug for empiric treatment if resistance is suspected.

P1156

Efficacy of daptomycin in the treatment of experimental endocarditis due to susceptible and multidrug-resistant enterococci

J.M. Entenza, M. Giddey, J. Vouillamoz, P. Moreillon *(Lausanne, CH)*

Objective: Antibiotic treatment of enterococcal infections may be difficult as most strains are multi-drug resistant. The aim of this study was to investigate the efficacy of daptomycin (DAP), a novel cyclic lipopeptide with excellent *in vitro* activity against resistant Gram-positive pathogens, in the treatment of rats with experimental endocarditis due to *Enterococus faecalis* and *E. faecium* with multi-drug resistant phenotypes.
Methods: The vancomycin-susceptible (VAN-S) E. faecalis JH2-2, its VAN-resistant (VAN-R) derivative (vanA phenotype) E. faecalis JH2-2/pIP819, and the VAN-R (vanB phenotype) and ampicillin-resistant (AMP-R) E. faecium D366 were used. MICs and time-kill studies were tested by CLSI (formerly NCCLS) method in Mueller-Hinton broth supplemented with 50 mg/L Ca++. Rats with catheter-induced sterile aortic vegetations were inoculated with 106 (E. faecalis) or 107 (E. faecium) CFU/ml. Treatment with simulated human kinetics of either DAP (6 mg/kg every 24 h), VAN (1 g i.v every 12 h) or amoxicillin (AMX; 2 g every 6 h) was started 16 h postinoculation and continued for two days. Control animals were sacrificed at the onset of therapy and treated rats 8 h after the trough level of the last antibiotic dose. Treatment outcome was assessed by counting the residual viable bacteria in vegetations.
Results: MICs for strains JH2-2, JH2-2/pIP819 and D366 were, respectively: DAP: 1, 1 and 2 mg/L; VAN: 2, > 32 and > 32 mg/L; AMX: 0.12, 0.12 and 4 mg/L. DAP was rapidly bactericidal *in vitro* against all strains at concentrations corresponding to peak (85 mg/L) and trough (10 mg/L) levels in serum. Killing was delayed, but not abolished, in the presence of 50% rat serum. The results of experimental endocarditis are shown in the table:

Strain	Phenotype	Infected rats/total			
		Controls	DAP	VAN	AMX
E. faecalis JH2-2	VAN-S; AMP-S	8/8	1/10	4/9	1/9
E. faecalis JH2-2/pIP819	VAN-R; AMP-S	9/9	2/11*	6/6	1/11
E. faecium D366	VAN-R; AMP-R	10/10	1/10* †	6/6	9/9

* $P < 0.05$ vs VAN † $P < 0.05$ vs AMX

Conclusions: In rats with experimental endocarditis, DAP, at doses simulating human kinetics of 6 mg/kg every 24 h, was significantly superior to VAN against VAN-R *E. faecalis* and *E. faecium*, and to AMX against AMP-R *E. faecium*. These results suggest that once-daily dosing of DAP may represent a therapeutic option for infections caused by multi-drug resistant enterococci.

P1157

Intracellular killing of *Staphylococcus aureus in vivo*: comparison of six antibiotics

S. Sandberg, R.L. Skov, N. Frimodt-Møller *(Copenhagen, DK)*

Objectives: Intracellular persistence of *S. aureus* has been acknowledged for years, but most reports deal with this issue in various cell-lines *in vitro*. For *S. aureus* few have studied the con-cept in experimental animal models *in vivo*. The purpose of this study was to develop an *In vivo* model to enable us to easily screen and diversify the extracellular as well as intracellular killing of different antibiotics.

Method: *S. aureus* E19977 (*pen*R and *methi*S) was used throughout. Antibiotics tested included di-cloxacillin, cefuroxime, gentamicin, rifampicin, clindamycin and azithromycin. MIC's were measured by Etest. Infection was induced by intraperitoneal injection of 5×10^{-7} CFU/ml in 5 % mucin in female NMRI mice. Two h later mice were treated with a single dose of antibiotic or saline for control. Four h later mice were killed, a peritoneal wash in 2 ml of saline was performed and the fluid retracted. The total bacterial count was quantified in the peritoneal wash. The cell fraction was isolated by centrifugation, and the extracellular bacte-rial count was quantified in the supernatant. The remaining extracellular bacteria in the pellet were killed by resuspension in HBSS containing 100 μg/ml of lysostaphin. The cells were lysed in cold sterile water, and the intracellular count quantified.

Results: The results are shown in the table as a reduction number which covers the number of re-ductions relative to the saline treated control

	Reductions in total	Reductions intracellular	Reductions extra cellular
Dicloxacillin	956	2040	979
Cefuroxime	212	1875	230
Clindamycin	25	13	50
Azithromycin	2.4	1.5	5.3
Gentamicin	8	3.5	18
Rifampicin	238	81	221,875

Conclusion: The intracellular results are surprising for the beta-lactams, since they are known for poor accumulation in cells. They even showed better results than rifampicin, which is known to be active both extra- and intracellularly. The poor reduction number seen for gentamicin intracellularly could be associated with its poor and slow penetration. Azithromycin and clindamycin reveal their bacteriostatic behaviour. In conclusion, the new *In vivo* model works satisfactorily and in future perspective, more complex pk/pd studies will be performed.

P1158

Efficacy of telavancin in the treatment of experimental endocarditis due to glycopeptide-intermediately susceptible *Staphylococcus aureus*

J.M. Miró, F. Marco, C. Garcia de la Maria, Y. Armero, E. Amat, D. Soy, A. Moreno, A. del Rio, M. Almela, C.A. Mestres, J. Gatell, M.T. Jimenez de Anta for the Barcelona Hospital Clinic Endocarditis Study Group

Background: Telavancin (TLV) is a novel, rapidly bactericidal lipoglycopeptide antibacterial with multiple mechanisms of action. Since the appearance of glycopeptide-intermediately susceptible *Staphylococcus aureus* (GISA), new alternatives to vancomycin (VAN) are needed. We evaluated the efficacy of TLV in experimental endocarditis in rabbits using two GISA clinical isolates: GISA ATCC 700788 and HIP5836.

Methods: 24 h after establishing catheter-induced aortic valve vegetations, an inoculum of 7×10^5 cfu/mL was injected IV. 18 h later the animals were treated for two days with either TLV (10 mg/Kg IV qd) or VAN (1gr iv bid) administered with a computer-controlled infusion pump system that simulated human serum kinetics. Control rabbits were sacrificed at 16 hours. Treated rabbits were sacrificed after completion of antibacterial therapy. Vegetations were quantitatively cultured.

Results: TLV and VAN MIC/MBCs were 1/4 and 2/2 mg/L for ATCC strain and 4/8 and 8/128 m/L for the HIP strain respectively. Peak and trough levels for TLV and VAN treatments were: 90 and 6 mg/L, and 46 and 6 mg/L, respectively.Therapy with TLV sterilized more vegetations that VAN. TLV reduced vegetations titers by 2.0 and 2.3 log greater than VAN for the ATCC and HIP strains, respectively, but the differences did not reach statistical significance ($p = 0.09$ and $p = 0.051$, respectively; Mann-Whitney rank sum test). All isolates from vegetations remained susceptible to TLV.

Treatment groups	#Survived/ # total (%)	#Sterile veg/ # total (%)	Median (IQR) log cfu/g veg
ATCC			
Control	-/-	0/17 (0)	9.5 (8.3 – 9.8)
VAN	20/23 (87)	4/20 (20)	6.6 (2.0 – 6.9)
TLV	16/17 (94)	6/16 (37)	4.6 (2.0 – 5.8)
HIP			
Control	-/-	0/20 (0)	9.1 (9.1 – 9.4)
VAN	15/15 (100)	1/15 (7)	6.7 (4.5 – 8.7)
TLV	16/17 (94)	5/16 (31)	4.4 (2.0 – 7.4)

Conclusions: After two days of therapy TLV was at least as effective as VAN in the treatment of GISA experimental endocarditis.

Bacterial pathogenesis-II

P1159

Alteration in virulence characteristics of plank tonic and bio film cells of *Pseudomonas aeruginosa* following exposure to macrophage secretory products

R. Mittal, S. Sharma, S. Chhibber, K. Harjai *(Chandigarh, IN)*

Objective: The primary line of innate defence against most bacterial pathogens consists of resident macrophages residing in tissues and polymorphonuclear neutrophils (PMNs) migrating from blood to the site of infection. Following stimulation of macrophages, secretory products are produced. The large array of bio molecules present in macrophage secretory products (MSPs) including pro as well as anti-inflammatory cytokines can influence ultimate outcome of an infection. The present investigation was planned to study the contribution of MSPs produced in response to interaction of macrophages with plank tonic and bio film cells of *P. aeruginosa* to virulence of this

pathogen in mouse model of ascending urinary tract infection. It has special relevance since receptor structures in mouse urinary tract are found to be similar to that of humans.
Method: Planktonic and bio film cells of clinical and standard strains of *P. aeruginosa* were exposed to murine peritoneal macrophages and macrophage secretory products were collected. These macrophage secretory products were characterized in terms of cytokine level, protein content and presence of reactive nitrogen intermediates and reactive oxygen species. Organisms were grown in presence of their respective MSPs and their pyelonephritic potential was assessed.
Result: Organisms grown in presence of MSPs were more virulent in vivo as indicated by significant increase in renal bacterial load, tissue pathology, malionaldehyde production and neutrophil recruitment.
Conclusion: The results of the present study bring out that pathogens poses mechanisms to exploit host defence mechanisms for its own survival leading to chronicity and recurrence of infections. Implications of these findings in relation to urinary tract infections (UTIs) caused by *P. aeruginosa* have been discussed.

P1160

Genetic analysis of alginate production in 140 cystic fibrosis *Pseudomonas aeruginosa* isolates from Scandinavian patients

O. Ciofu, T. Wassermann, U. Johansen, N. Høiby for the Scandinavian Cystic Fibrosis Study Consortium

P. aeruginosa adapts to the lung environment of patients with cystic fibrosis leading to chronic lung infection. One of the typical phenotypic changes is the occurrence of mucoid colony morphology, which indicates the overproduction of alginate. One mechanism of conversion to mucoidity is based on mutations in the algU/T mucABCD cluster, encoding the stress ó factor AlgT and its regulators. To investigate what kind of mutations is responsible for the alginate phenotype in Scandinavian CF patients, we sequenced algT, mucA, mucB and mucD genes in 70 pairs of mucoid/nonmucoid *P. aeruginosa* isolates with the same PFGE pattern. All patients were chronically infected and attended the CF Centres in Copenhagen and Aarhus (39 patients), Oslo (19 patients) and Uppsala (12 patients). The mean amount of alginate produced by the mucoid and nonmucoid *P.aeruginosa* isolates was 193 and 10.85 mg/L, respectively.
We found mutations in mucA in 93% of the mucoid and in 76% of the nonmucoid *P. aeruginosa* isolates. The most common mutations found were C/T transitions in the region 340–352 leading to premature stop codon (33.6 %), followed by mucA22 mutation (24.2 %), deletions (17.8 %) and insertions (6.4%) of isolates. The distribution of the C/T transitions in the region 340–352 leading to premature stop codon was 33%, 12% and 47% while the mucA22 mutation was found in 27%, 33.3 % and 13.5% in Denmark, Sweden and Norway, respectively. No deletions were found in the Swedish isolates and no insertions in the Norwegian isolates. The pulsed-field gel electrophoresis revealed that 15 Danish patients harboured a DK-1 clone, 8 patients a DK-2 clone and 13 Norwegian CF patients had a NO clone. The rest of 16 Danish patients, 6 Norwegian and all the 12 Swedish patients had non-clonally related strains. The distribution of the mucA mutation among the 68 non-clonally related *P. aeruginosa* isolates was muc A22 33.3%, C/T transitions 15.15% and deletions 15.15%. Mutations in mucA and algT, indicating that the nonmucoid isolates were revertants of the mucoid ones were found in only 37% of the nonmucoid isolates. Our findings show that the distribution of mucA mutations in CF *P. aeruginosa* isolates varies among the different Scandinavian CF centres and that besides the algT locus there are multiple pathways involved in the conversion from mucoid to nonmucoid phenotype.

P1161

Effect of different growth phases on surface properties and adhesion of *Pseudomonas aeruginosa*

A.P. Fonseca, C.I. Extremina, A. Freitas da Fonseca, J.C. Sousa *(Porto, PT)*

Objectives: *Pseudomonas aeruginosa* has been emerging as the primary source of nosocomial infections. Its pathological effects are attributed to many cell-associated virulence/survival factors. In clinical situations organisms can occur in different growth phases, which can alter the surface properties and adhesion abilities of the bacteria. The objective of this study was to evaluate the influence of different growth phases in the surface properties of *Pseudomonas aeruginosa* strains as well the effect in adhesion to polystyrene.
Methods: Six control strains, namely, 3 reference strains (ATCC 27853, PAO1, AK1), 3 defined PAO1 mutants with deviating surface characteristics (MT1562, PT623, PAO1algC) and 5 *P. aeruginosa* clinical isolates were used. The strains grown in LB were sampled during exponential and stationary phases. The effects of different growth phases on bacterial adhesion (1 h) were studied using a modified microtiter-plate assay. The changes on Microbial Adhesion to Solvents (MATS) were estimated by using a biphasic method, which calculates the percentage of cells adhering to hexadecane (a polar solvent), chlorophorm (acidic solvent and electron acceptor) and ethyl acetate (strongly basic solvent and electron donor).
Results: There was a significant increase in adhesion in the majority of the strains of *P. aeruginosa* from the exponential to the stationary growth phase. In bacterial adhesion to hexadecane (cell surface hydrophobicity) 6 strains had no differences between growth phases and 5 strains had the same results as adherence. Adhesion percentage to chlorophorm and to ethyl acetate was higher than to hexadecane, but there were no differences statistically significant in almost all the strains between the two growth phases. These two solvents, which have similar van der Waals (LW) properties and different acid-base characteristics, allow us to confirm the bipolar character of the bacterial strains studied. Our results suggest that the increase in adhesion values from exponential to stationary growth phase can be due mainly to changes in LW interactions.
Conclusion: There is an increase in adhesion values from exponential to stationary phase, partially explained, by the increase in cell surface hydrophobicity. The values with the two other solvents were regarded as a measure of the electron donor/basic and electron acceptor/acidic characteristics of the bacteria and confirm their bipolar character.

P1162

Effect of subinhibitory concentrations of ceftazidime and meropenem on the morphology and adherence ability of *Pseudomonas aeruginosa* wild-type strains

J. Vranes, S. Bekic, S. Ljubin-Sternak, B. Bedenic, N. Bauk, G. Mlinaric-Galinovic, L. Glavas-Obrovac *(Zagreb, Osijek, HR)*

Objectives: Antibiotics are often present at the site of infection at subinhibitory concentrations, because in human treated with

intermittent dosage schedules of antibiotics, suprainhibitory concentrations will always be followed by sub inhibitory levels. The aim of this study was to determine the effect of sub minimal inhibitory concentrations (subMICs) of ceftazidime and meropenem on the adherence ability of *Pseudomonas aeruginosa* strains isolated from a variety of isolation sites.

Methods: A total of 35 *P. aeruginosa* strains isolated from different clinical specimens (urine, bronchial secretion, and ear, throat and wound swabs) were used. The ability of strains to adhere to three different cell lines (Buffalo green monkey kidney, HeLa, and human fetal fibroblasts) was detected by immunofluorescence staining. Bacterial adherence to the cell line was tested before and after treatment with antibiotics. Comparison were made between the morphology before and that after exposure of strains to 1/2, 1/4, 1/8, 1/16 and 1/32 MIC of antibiotics, as well as between the number of bacteria attached to the cells before and the number after their exposure to the same concentrations of antibiotics.

Results: Most strains (89%) adhered well to the used cell lines, and there was no significant difference between adherences of the strains to the three cell lines used. Upon comparison of the number of attached bacteria before and after exposure to subMICs of antibiotics, statistically significant difference was determined after exposure of the strains to 1/2, 1/4, 1/8 and 1/16 MIC of ceftazidime, and after exposure of the strains to all the concentrations of meropenem used. SubMICs of antibiotics alter not only bacterial adherence ability but also their morphology. Morphological changes were most prominent after bacterial exposure to 1/2, 1/4 and 1/8 MIC of ceftazidime and meropenem. Higher concentrations of those antibiotics caused formation of very long filaments with irregular swellings. A very high correlation was observed between the changes in the length of bacteria and their loss of adherence ability.

Conclusion: The alteration in bacterial morphology caused by subMICs of ceftazidime and meropenem was related to subsequent loss of bacterial adherence ability.

P1163

In vitro study of the influence of phenyl lactic acid produced by lactobacillus probiotic strains on the pathogenic features of *Pseudomonas aeruginosa* strains

V. Lazar, L. Ditu, M.C. Balotescu, C. Bleotu, M. Bucur, O. Dracea, O. Banu, R. Cernat *(Bucharest, RO)*

Introduction: Our previous studies concerning the probiotic activity of some Lactobacillus strains have been shown that they possess inhibitory activity toward the growth of pathogenic bacteria and the biochemical analysis of the organic acids profile showed that the probiotic strains produced phenyl lactic acid (PLA) in significant amounts. The purpose of this study was to investigate the influence of PLA on the rate growth, the expression of enzymatic virulence factors, antiobiotic resistance markers and adherence capacity to the cellular and inert substrata of some *P. aeruginosa* strains recently isolated from bio film-forming infections.

Material and methods: Seven 7 *Ps. aeruginosa* strains isolated from central venous catheter pieces and from tracheal and broncho-pulmonary secretions were cultivated in liquid nutrient broth in the presence of different concentrations of PLA (dissolved in methanol: water 1:1). The microbial cultures were harvested at different times of the bacterial growth curve (1, 2, 3, 4 and 24 h) and investigated for the expression of different enzymatic factors (haemolytic activities, caseinase, gelatinase, amylase, lecithinase, lipase, mucinase), adhesion and invasion of the cellular substrate represented by HeLa cells (Cravioto's adapted method), adhesion to inert substrata represented by central venous catheter pieces and antibioresistance markers (disk diffusion method). The influence of PLA on bacterial cell viability was assayed by viable cell counting using the calibrated loop method on agar and by spectrophotometric assesment of the microbial culture density.

Results: PLA influenced the growth rate of the bacterial cells, in a dose dependent manner. The PLA did generally not affect the pattern of soluble enzymatic factors secretion at different incubation times (haemolytic activities, lipase, caseinase) with one exception, i.e. the activation of DNA-se secretion in logarithmic phase cultures in the presence of PLA, while the control culture became positive in the stationary growth phase. It is to be noticed that PLA induced morphological changes of the bacterial cells, suggesting its influence on the expression of bacterial cell surface-associated molecules. The influence of PLA on the adherence capacity to the cellular and inert substrata was either inhibitory or stimulatory, depending on the tested strain. The expression of antibiotic resistance mechanisms in the tested strains was not affected.

P1164

Bloodstream infections due to *Pseudomonas aeruginosa*: clinical outcome associated with pathogenesis-related genes

A. Endimiani, B. Pini, A. Baj, F. Luzzaro, A. Toniolo *(Varese, IT)*

Objectives: *P. aeruginosa* (Pa) overcomes host defence mechanisms using two main protein secretion systems: a) type II system involved in the production of exotoxin A (exoA), elastases (lasA and lasB), and phospholipase C (plcH and plcN); b) type III system implicated in the production of exoenzymes (exoS and exoT), phospholipase A (exoU), and adenylate cyclase (exoY). Neuroaminidases (nan1 and nan2) play an important role in Pa adherence to epithelial cells. This study was planned to evaluate whether pathogenesis-related genes were linked to clinical features and outcome of patients with Pa-induced bloodstream infection (BSI).

Methods: Thirty-eight Pa isolates causing BSI at our institution were studied. Twenty strains had a multi-drug resistant (MDR) phenotype. Real time PCR (Applied Biosystems, Foster City, CA) was used to evaluate the presence of exoS, exoT, exoU, exoY, lasB, plcH, plcN, exoA, nan1, and nan2. Clinical records of BSI-patients were examined retrospectively. Age, length of hospital stay, underlying diseases (McCabe-Jackson classification and Charlson weighted index), severity of sepsis, primary source of infection, and clinical outcome were evaluated. Statistical analysis was performed using the Statistica software (StatSoft, Tulsa, OK).

Results: 38/38 Pa isolates were positive for the following genes: exoT, exoU, lasB, plcH, plcN, exoA, and nan2. The remaining exoS, exoY and nan1 genes were detected in 30 (78.9%), 28 (73.7%) and 22 (57.9%) isolates, respectively. Independent statistical analysis showed that the MDR phenotype was associated with exoS and nan1 genes ($P = 0.02$ and $P < 0.01$, respectively). Rapidly fatal underlying disease was associated with the exoS gene ($P = 0.04$). No significant differences were observed with regard to the exoY gene. Overall, 22/38 (57.9%) isolates were positive for the entire type III secretion pathway. Statistical analysis showed that no significant clinical and microbiological differences were observed regarding the type III system.

Conclusions: Compared to published data, the results show that genotypes of Pa strains causing BSI are similar to those determining pneumonia in CF patients. Notably, two central

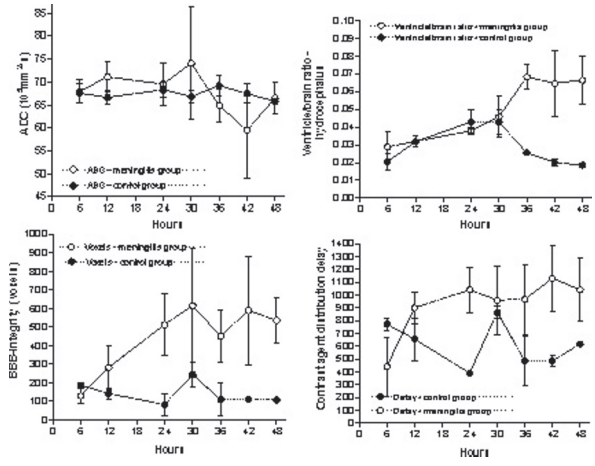
pathogenetic genes (exoS and nan1) were frequently associated with MDR strains. The finding suggests that Pa isolates showing MDR traits may be more virulent than susceptible strains.

P1165

MR imaging in experimental meningitis – *in vivo* correlates for disease severity

C.T. Brandt, H. Simonsen, J.D. Lundgren, C. Østergaard, N. Frimodt-Møller, L.V. Søgaard, O.B. Paulsson, I. Rowland *(Copenhagen, DK)*

Background: Breakdown of the blood-brain-barrier (BBB), alterations in cerebral blood supply, oedema of the brain and ultimately hydrocephalus and nervous tissue damage are factors believed to be associated with disease severity of *pneumococcal meningitis*. The developmental relations between these deleterious entities of disease remains to be defined.
Materials/Methods: Meningitis was induced by intracisternal injection of ~1 x 105 CFU/ml *S. pneumoniae* serotype 3 (n = 29) or saline (n = 13). Rats were randomised and sacrificed (4 infected 2 saline inoculated at each time) after 6,12,24,30, 36,42 and 48 h post-infection and brains harvested for histopathology. Prior to imaging rats were subject to a clinical and neurological score, and cerebrospinal fluid and blood samples obtained. MR images were acquired using a SISCO 4.7T imaging system. T1W, T2W, quantitative diffusion, dynamic MRI and post contrast (0.5 mmol/kg GdDTPA). Brain oedema was measured with ADC maps calculated from images acquired with b-values of 0, 185, 740 and 1665 s/mm^2. Blood-brain barrier integrity and perfusion delay was investigated by dynamic MRI and gadolinium enhancement kinetics. Ventricular size was an accurate measure of the mean ventricle-brain ratio in 3 coronal MR images.
Results: Meningitis was confirmed by pneumococcal growth in the CSF and the increasing CSF pleocytosis. Rats with meningitis expectedly differed highly significantly from saline inoculated controls in all examined parameters: WBC counts, clinical score, motor score, ventricle-brain (v-b) ratio, brain oedema, BBB-integrity, cerebral perfusion and ADC (two-way ANOVA, $P < 0.0001$). Increasing ventricle-brain ratio was found to be the closest correlate to clinical disease and motor performance scores (spearman rank rho = 0.83 and rho = 0.82, $P > 0.0001$, respectively). The presence of cerebral damage was associated with more extreme values of the examined parameters.



Conclusion: The course of disease was predictable with severe and terminal illness developing within 36 to 48 hours after infection. Twelve hours after infection BBB integrity was reduced and associated with increased indices of vasogenic brain oedema and reduced cerebral perfusion. Severe clinical deterioration developed from 30 h onwards and was associated with increased ventricular size, decreased ADC (cytotoxic oedema or cortical compression) and a further decrease in perfusion whereas BBB integrity, CSF infection and pleocytosis reached a maximum at 30 h.

P1166

Transmission of community-acquired methicillin-resistant *Staphylococcus aureus* infection in household contacts in Taiwan

Y-C. Huang, C-F. Ho, C-J. Chen, L-H. Su *(Kweishan, Taoyuan, TW)*

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a community-acquired (CA) pathogen recently. However, its transmission in households and the community has not been thoroughly investigated.
Methods: Prospective family cohort study was conducted to investigate children and household members of these patients who had documented CA-MRSA infections between August 2004 and May 2005. 57 case patients and 121 household members underwent a survey for nasal carriage of MRSA. Microbiological characteristics of the infected and colonized MRSA isolates including pulsed-field electrophoresis (PFGE), staphylococcal chromosomal cassette (SCCmec) type, and Panton Valentine leukocidin (PVL) genes were determined and compared. Some strains of representative PFGE patterns were selected for multilocus sequence typing (MLST).
Results: Of the 57 families with 121 household contacts sampled, MRSA was detected in 30 contacts (25%) from 23 families (40%). 54 clinical isolates from 48 case children were available for analysis and showed that genotypes D (34/48, 71%) and C (7/48, 15%) were the two most common PFGE patterns, particularly type D-SCCmec VT-PVL (+) (in 32 children) and type C-SCCmec IV-PVL (−) (in 7). Both the infected and household colonized isolates were indistinguishable in 64%, highly related in 18% and distinct in 18% of 28 pairs of isolates available for comparison. Nasal carriage of MRSA was noted in 18 (32%) of the 57 case patients and both the infected and colonized isolates were indistinguishable in 94% and highly related in 6% of 16 pairs. MLST was performed in 14 isolates, disclosing that ST59 was identified in 8 isolates of types D (4), C (3), and AA, ST338 (single locus variant of ST59) in 2 isolates of type D, and ST89, ST8, ST239 and ST30 in one isolate each.
Conclusion: A predominant clone of MRSA (ST59, SCCmec VT, PVL (+)) was identified from the CA-MRSA isolates in Taiwanese children. For children with CA-MRSA infection in Taiwan, a substantial proportion of these children and their household members are colonized with an indistinguishable strain.

P1167

Characterisation of Panton–Valentine leukocidin-positive *Staphylococcus aureus* strains isolated in the Czech Republic

P. Petras, I. Machova, M. Marejkova, R. Pantucek, V. Ruzickova, J. Doskar *(Prague, Brno, CZ)*

Objectives: Here we report the first Panton–Valentine Leukocidin (PVL) positive *S. aureus* strains isolated in the Czech Republic after their emergence in many European countries, USA and Australia.
Methods: Carriage of the lukS – lukF genes for PVL was detected by previously described PCR primers. The RPLA

method (Denka Seiken) was used for detection of enterotoxin A, B, C, and D types, TSST-1, and exfoliative toxin A, and B production. PCR detection of the tst, eta and etb genes allowed confirmation of toxigenicity. Resistance to 12 antibiotics was tested by disk diffusion methods (Oxoid). Phage typing was performed by the standard method using the International Set of Phages (PHLS London). The MRSA PVL positive strains were characterized by SmaI macro restriction analysis resolved by PFGE, SCCmec typing by a multiplex PCR assay and prophage typing assay and preliminary characterization of prophage types concerning to PVL production.

Results: The presence of the lukS–lukF genes was detected in 34 (7.7%) of 443 *S. aureus* strains isolated mainly from deep-seated skin infections and referred to the NRL for Staphylococci, NIPH, Prague, from Czech microbiological laboratories. Production of enterotoxins A–D was found in 12 (35%) of the 34 PVL positive strains. Three strains also produced TSST-1; one strain was a producer of exfoliative toxin A. Only 4 of PVL positive strains were resistant to oxacilin. Those MRSA strains were hospital acquired, and were multiresistant. Four MRSA strains were characterized by molecular methods and correlated with the known European MRSA types. A sizeable part of PVL positive MSSA isolates were resistant to erythromycin (60%), amoxicillin/ clavulanate (52%), and clindamycin (36%). Resistance to other antibiotics was rare.

Conclusions: Only 4 multiresistant and hospital acquired strains of 34 (12%) Czech PVL positive isolates showed resistance to oxacillin. Most PVL positive strains were phage–typeable and were predominantly classified into phage group II (70%).

Acknowledgements: This work was supported by research programme No. MSM0021622415 of the Ministry of Education, Youth and Sports of the Czech Republic.

P1168

Increased incidence of higher vancomycin minimal inhibitory concentration and changing pattern of antibiotic susceptibility in methicillin-resistant *Staphylococcus aureus* isolates: a single institution study

D. Lu, P. Holtom *(Los Angeles, US)*

Background: The rate of methicillin-resistant *Staphylococcus aureus* (MRSA) infections at our institution is 50–60% of all *Staphylococcus aureus* infections, thanks to the recent community-acquired MRSA (CA-MRSA) epidemic. Yet little is known about changes in the antibiotic susceptibility pattern of these strains. Has CA-MRSA replaced the traditional hospital-acquired MRSA to become the new hospital flora, as manifested by a new pattern of antibiotic susceptibility? Another growing concern is glycopeptide heteroresistance. The incidence of MRSA with higher vancomycin minimal inhibitory concentration (vanc-MIC) is unknown. We sought to address these two issues in our epidemiologic study.

Methods: Microbiology data for all MRSA isolates at our hospital from September 1, 2004 to March 1, 2005 were analysed. Sample location and isolates' antibiotic susceptibility and vanc-MIC were compiled.

Results: Over the 6 months period, 1023 MRSA were isolated. Nasal cultures were not included and recurrent infections counted only once. Only 32 (3%) of the 1023 isolates were found to be resistant to 3 or more antibiotics, in addition to the beta lactams. 188 isolates (18.4%) were found to have a vanc-MIC of 2. There were no isolates with vanc-MIC greater than 2. Of those 188.69 (36.7%) came from patients in the emergency department or primary care clinics. Only 9 (4.8%) of the 188 isolates were found to be multi-drug resistant.

Conclusions: Our study demonstrates that, at our institution, MRSA antibiotic susceptibility resemble that of CA-MRSA. Higher vancomycin MIC among MRSA is seen with increasing frequency. Follow-up studies are needed to determine the clinical significance of these findings.

P1169

Prevalence of different virulence factors in enteroaggregative *Escherichia coli* isolates causing diarrhoea in children in Ifakara, Tanzania

E. Mendez Arancibia, J. Ruiz, M. Vargas, J. Gascon, J. Vila *(Barcelona, ES)*

Objectives: The pathogenesis of enteritis caused by enteroaggregative *Escherichia coli* (EAEC) remains under discussion. To date several virulence factors (VF) have been established in EAEC. The main aim of this study was to determine the prevalence of 18 VF in 86 EAEC isolates causing diarrhoea in children under five years of age from Ifakara, (Tanzania).

Methods: EAEC strains were detected using a PCR validated method and by adherence assay. The differentiation between typical and atypical EAEC was based on the presence of the aggR gene, also detected by PCR. In both groups of EAEC strains, the following genes were detected by PCR: lt gene (heat-labile toxin), east gene (heat-stable enterotoxin), shet gene (Shigella enterotoxin 1 and 2), aspU gene (plasmid-encoded cryptic secreted protein), sat gene (autotransported toxin), cdtB gene (cytolethal distending toxin), pet gene (the plasmid encoded toxin), aafI and aafII (adherence factors), eaf gene (enterophatogen adherence factor), bfp gene (bundle-forming pili), fyuA gene (yersiniobactin), Ag43 (Antigen 43), eae gene (intimin), ipaH gene (invasion antigen gene of enteroinvasive *E. coli* EIEC) and the stx gene (verotoxins VT1 and VT2), being used. The Fishers exact test and the Chi-squared test were used to analyse the results obtained.

Results: From these 86 isolates, 50 (58.1%) presented the aggR gene (being classified as typical EAEC) while 41.9 % did not. This difference was not statistically significant. Overall, 70% and 66.7% of the typical and atypical EAEC showed at least one of the studied VF. The median of VF among the analysed strains was of 1.73. Twenty-six percent of the EAEC isolates did not show any of the VF tested. Ag 43 (30.6%) and Aspu (29.5%) were the most frequently found VF, followed by Sat (21.2%), Shet1 (20%), FyuA (18.8%), EAST (16%) and CdtB (11.8%). The remaining VFs were detected in less than 10% of the strains.

Conclusion: These results suggest the possible presence of unidentified factors that may underlie the virulence of these EAEC strains. Moreover, these results show the high heterogeneity of EAEC isolates and highlight the potential role of Ag43 and Sat as VF of EAEC.

P1170

Profound difference in virulence genes carriage between Pakistani and Swedish *Escherichia coli* strains belonging to phylogenetic group B2

F.L. Nowrouzian *(Göteborg, SE)*

Objectives: *E. coli* strains segregate into four major phylogenetic groups, termed A, B1, D and B2. Group B2 strains cause most extra-intestinal infections, carry the largest number of virulence-associated genes and have an increased

capacity to persist in the colonic micro biota. This has, however, almost exclusively been demonstrated in bacteria sampled from Western countries. In this study we examine phylogenetic group distribution in relation to virulence gene carriage and colonic persistence of *E. coli* obtained from Pakistani infants.
Methods: *E. coli* strains sampled from colonic microbiota of 22 Pakistani infants followed longitudinally over the first six months of life. Individual strains were identified by multilocus enzyme electrophoresis. Strains appearing on more than one occasion were defined as resident, while strains isolated on a single occasion only were defined as transient. The strains were characterized with respect to carriage of several virulence-associated genes and were tested for phylogenetic origin using triplex PCR (polymerase chain reaction).
Results: Overall 158 *E. coli* strains were identified in the Pakistani infants. Forty seven percent belonged to phylogenetic group A, 23% to group D, 18% to B1 and 12% to B2. The strains belonging to phylogenetic group B2 significantly more often carried the genes for fimA ($p = 0.003$) compared to other strains. As compared to Swedish group B2 *E. coli* strains, Pakistani B2 strains lacked the genes sfaD/E (S, F1C fimbriae), neuB (K1 antigen) and papG class III and they significantly less often carried genes for papC ($p = 0.02$) and hylA ($p = 0.005$). Further, in contrast to previous finding in Swedish *E. coli* B2 strains, group B2 strains from Pakistani infants were no more likely to persist in the intestinal micro biota than strains from the other phylogenetic groups.
Conclusion: The results suggest that *E. coli* group B2 strains from Pakistani infants differ substantially from Western group B2 strain. Their lower carriage rate of various virulence factor genes may explain their inferior capacity to persist in the micro biota and suggest that the accumulation of virulence genes in B2 strains has occurred after the subdivision of *E. coli* strains into four phylogenetic groups.

P1171

Pathogenicity island I of *Escherichia coli* CFT073 and Yersinia high-pathogenicity island are significantly associated with persistence of *E. coli* in the intestine of Swedish infants

A.E. Östblom, I. Adlerberth, A.E. Wold,
F.L. Nowrouzian *(Göteborg, SE)*

Objectives: Virulence associated genes are often located on particular regions on the bacterial chromosome, termed pathogenicity islands. Uropathogenic *Escherichia coli* strains often carry pathogenicity islands, such as the pathogenicity island I (PAI ICFT073) first identified in the uropathogenic *E. coli* strain CFT073 and the high-pathogenicity island (HPI) originally found in Yersinia species. We have examined whether these pathogenicity islands may contribute to persistence of *E. coli* in its normal niche, the colonic microflora.
Methods: *E. coli* obtained from 70 Swedish infants followed with regular sampling of the faecal flora over the first year of life were analyzed. The *E. coli* isolates were been identified to the strain level by Random Amplified Polymorphic DNA (RAPD). A total of 148 strains were classified, of these 58 strains were resident (persisting for ≥ 3 wk in the microflora) and 19 could be classified as transient (persisting for ≤ 3 wk in the micro flora). The strains were assessed for carriage of PAI ICFT073 and HPI, by the use of a single PCR and a duplex PCR assay, respectively.
Results: Fifty six and 47% percent of the *E. coli* strains harboured HPI and PAI ICFT073 respectively. Resident strains significantly more often than transient strains possessed PAI ICFT073 (66% vs. 21% $p = 0.001$) and HPI (70% vs. 37% $p = 0.01$).
Conclusion: Our results indicate that HPI and PAI ICFT073 enhance the colonization capacity of *E. coli* strains in the intestine. The virulence associated traits characterizing resident *E. coli* strains may contribute to the persistence in the intestinal micro flora as well as increase their pathogenic capability in the urinary tract.

P1172

Relationships between phylogenetic groups of *Escherichia coli* isolates and clinical characteristics in 161 patients with bacteraemia

F. Jaureguy, E. Carbonnelle, S. Bonacorsi, E. Bingen, B. Picard, X. Nassif, O. Lortholary *(Bobigny, Paris, FR)*

Objectives: The aim of this study was to assess the relationship between phylogenetic groups of *Escherichia coli* strains isolated from bacteraemia and clinical characteristics of the sepsis.
Methods: A prospective study was conducted between December 2002 and December 2003 on 161 patients (including 14 children) with *E. coli* bacteremia in Necker and Avicenne University Hospitals, Paris, France. Clinical data included primary source of bacteremia, underlying diseases including any immunodeficiency, hospital versus community acquired origin and outcome. Bacteriological data included determination of phylogenetic groups performed by triplex PCR (chuA, yjaA, DNA fragment TSPE4) and antibiotic susceptibility to amoxicillin (AMX), ofloxacin (OFX) and trimethoprim-sulfamethoxazole (STX).
Results: Among the 161 case patients, 51 % were older than 65 years, 36% were immunocompromised and their severity sepsis scoring according to Bone's criteria was: Stage 2 (81.4 %), Stage 3 (7.5 %) and Stage 4 (11.5 %). Of the 161 *E. coli* blood infections, 38. 5% were of nosocomial origin. The most frequent source of bacteremia was the urinary tract (41 %) followed by the digestive tract (10.6%). The 161 strains were distributed into phylogenetic groups B2 (49.7%), D (22.4 %), A1 (21.7 %) and B1 (6.2 %). Sixty three percent of these isolates were resistant to AMX, 38 % to STX and 17 % to OFX. A factorial analysis of correspondence conducted on the data allowed the distinction of 2 groups of strains. The strains belonging to the phylogenetic group B2 were more frequently associated to the following characteristics: susceptibility to the 3 tested antibiotics, community acquired infection, urinary tract origin, immunocompetent host and favourable outcome. The strains belonging to the phylogenetic groups A, B1 and D were more frequently associated to: resistance to the 3 tested antibiotics, hospital acquired infection, non urinary tract origin, immunocompromised host, Stage 4 and higher mortality.
Conclusion: Results distinguished: (i) urosepsis due to B2 group susceptible strains infecting immunocompetent patients with favourable outcome, (ii) bacteraemia of others sources due to non-B2 group resistant strains infecting older and immunocompromised patients with severe outcome.

P1173

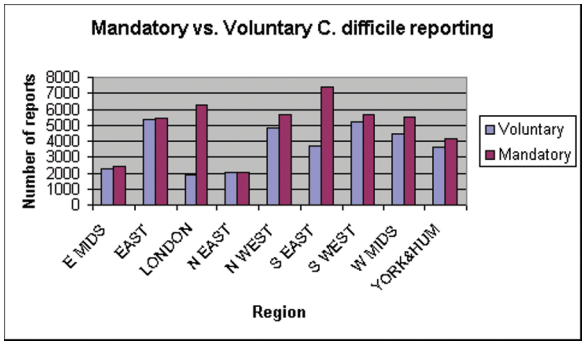
Burden of *Clostridium difficile* associated disease in NHS hospital trusts in England

A.D. Pearson, K. Wagner, A. Talebi, E. Robinson,
L. Saker *(London, UK)*

Summary statement: Results of the first year of mandatory *C. difficile* reporting are compared to the results and trends observed in the national voluntary reporting system during the period 1990 to 2004.
Methods: Surveillance of *Clostridium difficile* associated disease (CDAD) has been included in the mandatory healthcare-

associated infection surveillance system for NHS acute Trusts in England since January 20041. Data are collected quarterly from the 169 acute Trusts in England that treat patients aged 65 years and over. The findings from the first year of mandatory surveillance are compared with the results from the national voluntary system of reporting and used to estimate the current burden of CDAD and assess the national trend. For the purposes of the mandatory surveillance scheme, microbiology laboratories were required to test diarrhoeal specimens for evidence of CDAD from all patients over 65 years old who have not been diagnosed with CDAD in the preceding four weeks. Diarrhoeal stools are defined as those that take the shape of their container. Non-diarrhoeal stools should not be tested for CDAD. Laboratories should test specimens for *C. difficile* toxin using either an immunoassay detecting both toxin A and toxin B, or a neutralised cell cytotoxicity assay. The method used should be subject to appropriate quality assurance. The mandatory surveillance scheme does not distinguish between hospital and community-acquired cases. Even cases considered to be community-acquired should be reported by the Trust in which they are detected.

Results: During the period January-December 2004, the HPA received 44,488 reports of CDAD from 166 out of 169 acute NHS Trusts (two Trusts did not submit any data, and one Trust did not submit data for three quarters). Four Trusts had no cases to report. In contrast the national voluntary reporting system indicated that there were 33,493 cases in patients aged 65 years and above. The relative ascertainment in the nine regions is given in the attached figure. This indicates that the under ascertainment of the voluntary surveillance for CDAD was 32% in 2004. These findings will be applied to the 15 year analysis of trend.



Conclusion: The total number of CDAD reports for all age groups was 44,551 in the voluntary scheme. Applying the under ascertainment factor seen for the selected age group in the mandatory suggests the total burden of cases for all age groups in 2004 was 58,807 cases.

P1174

In vitro investigation of pro-inflammatory properties of *Helicobacter pylori* strains involved in low-grade gastric mucosa associated lymphoid tissue lymphoma

P. Lehours, G. Lasne, A. Gallois, A. Menard,
A. Ruskone Fourmestraux, J.C. Delchier, F. Megraud *(Bordeaux, Paris, Creteil, FR)*

Objectives: In a previous study, we studied the main *H. pylori* virulence factors in strains isolated from patient with low grade gastric mucosa associated lymphoid tissue (MALT) lymphoma, and showed that none can be associated to these strains (Lehours et al., Infec Immun. 2004; 72:880–8). We then postulated that MALT pathogenesis was not linked with more pro-inflammatory *H. pylori* strains. Nevertheless, the assumption that these strains possessed unknown pro-inflammatory factors lacked confirmation. We investigated the ability of 33 *H. pylori* MALT strains and the two *H. pylori* sequenced strains J99 and 26695 to induce interleukin-8 (IL-8) secretion by the human gastric adenocarcinoma epithelial AGS cell line.

Methods: *H. pylori* strains were harvested from 2 days plate cultures then resuspended in brucella broth. The broth was adjusted to an optical density of 0.6 at 600 nM corresponding to approximately 3 x 107 CFU/ml. The bacterial suspensions (35 μl) were inoculated in each well in triplicate on AGS cells cultured to 60–70% confluence in F12K/10% FBS medium. After 18 h of co culture at 37°C, the supernatants were recovered in order to quantify immediately or after freezing the pro-inflammatory cytokine IL-8 (Quantikine Human IL-8 Immunoassay, R&D Systems).

Results: IL-8 levels obtained for the reference strains (J99 and 26695) were in line with the values published in the literature for this *in vitro* model. Because IL-8 production is dependent on a functional cag secretion system, we studied 16 cagA positive and 17 cagA negative MALT strains. High IL-8 production was observed in the cagA positive strains (1379 pg/ml + 659). All of the negative cagA strains induced low rates of IL-8 (75.2 pg/ml + 39.7), which indicates that they do not have a pro-inflammatory potential nor other important pro-inflammatory factors.

Conclusion: Although the involvement of *H. pylori* strains in MALT lymphoma is well established, gastric inflammation, especially for cagA negative strains representing 50% of them, does not seem to be the pathomechanism of this cancer. We are currently testing more MALT lymphoma strains. We are also analysing or results according to the presence of the main *H. pylori* virulence factors and putative virulence genes identified in the laboratory.

P1175

Reduced activation of microglial cells by *Streptococcus pneumoniae* after treatment with nonbacteriolytic antibiotics in comparison to ceftriaxone

A. Spreer, I.M. Hernekamp, M. Lotz, S. Ebert, H. Eiffert,
R. Nau *(Gottingen, DE)*

Objective: Neuronal injury in pneumococcal meningitis is a consequence of the direct toxicity and proinflammatory activity of bacterial components. Antibiotic standard therapy consists of highly effective bacteriolytic beta-lactam antibiotics. However, beta-lactam antibiotics act by bacterial lyses resulting in an increased release of bacterial products.

Methods: Primary cultures of mouse microglial cells and primary mouse cortex neurons were exposed to culture supernatants of *Streptococcus pneumoniae*, grown in tryptic soy broth without addition of antibiotics or after treatment with non-bacteriolytic antibiotics (clindamycin, rifampin) or ceftriaxone.

Results: Primary mouse microglial cells were activated by culture supernatants of *Streptococcus pneumoniae*, while no direct toxic effect of pneumococcal culture supernatants on primary neurons was detected. Treatment of pneumococcal cultures with clindamycin or rifampin in comparison to ceftriaxone significantly reduced the proinflammatory action of culture supernatant on microglial cells. This effect was also present after sequential therapy with nonbacteriolytic and bacteriolytic antibiotics.

Conclusion: During bacterial meningitis, the occurring neuronal damage is in parts ascribed to microglia-mediated toxicity. *In vitro*, the extent of microglial activation by pneumococcal culture supernatants is reduced by the usage of non-bacteriolytic antibiotics in comparison to beta-lactams. Therefore antibiotic regimens relying on bactericidal protein synthesis inhibitors should be evaluated for meningitis therapy.

P1176

Contribution of chemokines CXCL10 and CXCL11 and their receptor CXCR3 to the accumulation of memory CD4 + T-cells in the cerebrospinal fluid of patients with tick-borne encephalitis and neuroborreliosis

S. Zidovec Lepej, O. Dakovic Rode, L. Misic Majerus, B. Barsic, A. Vince, T. Jeren *(Zagreb, Koprivnica, HR)*

Objectives: The recruitment of peripheral blood (PB) cells into the cerebrospinal fluid (CSF) is mediated by chemokines and their corresponding receptors. The aim of this study was to compare the concentrations of CXCL10 and CXCL11 in the CSF and serum of patients with tick-borne encephalitis (TBE) and neuroborreliosis and to evaluate the contribution of these chemokines to the recruitment of CXCR3-expressing CD4 + T-cells into the CSF.

Results: Levels of CXCL10 in the CSF of neuroborreliosis (median 733.3 pg/mL) and TBE (median 365.1 pg/mL) were significantly increased compared with serum (median 155.4 pg/mL and 71.7 pg/mL, respectively, $p < 0.001$ for both groups). Contrary to this finding, CSF of NIND patients contained significantly lower concentration of CXCL10 median 9.2 pg/mL) compared to the serum (median 98.5 pg/mL, $p < 0.001$). Concentration of CXCL11 in the CSF of neuroborreliosis patients (median 17.3 pg/mL) was significantly increased compared with serum (median 6.8 pg/mL, $p < 0.001$). Detectable levels of CXCL11 (> 13.9 pg/mL) were found in only 4 CSF and 3 serum samples of TBE patients. CXCL11 concentration in the CSF of NIND patients (median 69.8 pg/mL) was significantly increased compared with serum (median 8.9 pg/mL, $p < 0.001$). Percentages of CXCR3-expressing memory CD4 + T-cells in the CSF were higher compared to the PB in both patient groups.

Methods: This prospective, cross-sectional study included 20 neuroborreliosis patients with early CNS involvement proven by intrathecal synthesis of antibodies to B. burgdorferi s.l., 16 patients with TBE and 11 patients with non-inflammatory neurological diseases (NIND). Chemokine receptors on CSF and PB T-cells were analysed by flow cytometry. CXCL10 and CXCL11 in the CSF and serum of patients were quantified by commercially available enzyme-immunoassays.

Conclusion: Our results suggest that CXCL10 and CXCL11 create a chemokine gradient between the CSF and serum and are, in part, responsible for the recruitment of CXCR3-expressing memory CD4 + T-cells into the CSF of patients with both neuroborreliosis as well as TBE.

P1177

Assessment of monocytic function in patients with septic syndrome induced by ventilator-associated pneumonia

T. Tsaganos, M. Raftogiannis, D. Zervakis, D. Plachouras, C. Skalioti, S. Kollias, E.J. Giamarellos-Bourboulis, C. Roussos, H. Giamarellou *(Athens, GR)*

Objectives: The present study aimed to investigate the impact of VAP-induced sepsis on monocytic function.

Methods: Ninety patients presenting with VAP-induced-sepsis were enrolled in the study and divided into three groups according to the severity of the septic syndrome; patients presenting with sepsis, patients with severe sepsis and patients with septic shock. Blood was drawn on the day of enrolment and on the third, fifth and seventh day thereafter. For comparison, blood was also collected from five healthy volunteers. The collected blood was layered over Ficoll and centrifuged. Isolated mononuclear cells were incubated in RPMI for one hour; non-adherent cells were removed and monocytes harvested after trypsinization. They were then distributed in two wells of a 12-well plate and incubated for 18 hours with and without endotoxin (LPS). Supernatants were collected, aliquoted and refrigerated pending assay. TNFa and IL-6 concentrations were estimated by an enzyme immunoassay. The function of monocytes was determined by difference of estimated concentrations of cytokines between monocytes incubated with endotoxin and monocytes incubated without endotoxin.

Results: Mean concentration of TNFa produced by monocytes of controls incubated without LPS was 38.31 pg/10000cells and of IL-6 was 7.90 pg/10000cells. Respective concentration of TNFa by LPS-induced monocytes of controls was 545.59 pg/10000cells and of IL-6 was 90.88 pg/10000cells. Mean concentration of TNFa and IL-6 produced by LPS-induced monocytes of patients with ventilator-associated pneumonia is shown in the table.On days one, five and seven, TNFa production by LPS-stimulated monocytes is statistically reduced in all three groups compared to controls, whereas on day three, only patients with sepsis and septic shock present with reduced TNFa production. Production of IL-6 by LPS-stimulated monocytes differs statistically among groups on the first day and between controls and patients with severe sepsis and septic shock on the fifth day.

Day	Without endotoxin			Endotoxin induced		
	Sepsis	Severe Sepsis	Septic Shock	Sepsis	Severe Sepsis	Septic Shock
	Mean TNFa concentration (pg/10000 cells)					
1	80.03	41.08	66.81	242.10*	113.02*	154.64*
3	129.36	33.05	23.91	101.86*	389.24	95.97*
5	32.43	28.01	24.18	79.83*	132.94*	89.41*
7	61.18	43.65	94.24	164.08*	232.75*	133.42*
	Mean IL-6 concentration (pg/10000 cells)					
1	44.56	26.07	49.39	168.25*	50.86*	734.00*
3	41.55	27.54	26.80	147.18	77.36	89.32
5	34.08	26.47	38.06	96.44	56.13*	151.98*
7	34.37	23.70	50.64	122.49	59.91	100.70

*Denotes significant difference between group and control

Conclusion: TNFa production by LPS-stimulated monocytes is reduced in all patients presenting with septic syndrome induced by VAP on all days; impaired monocytic function results in reduced IL-6 production on first and fifth days. Monocytic function in patients with sepsis by ventilator-associated pneumonia is impaired upon start of septic syndrome and improves slightly over the course of time.

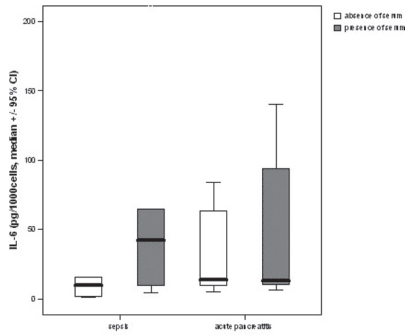
P1178

Monocytes in systematic inflammatory response syndrome: differences between sepsis and acute pancreatitis

F. Baziaka, T. Tsaganos, V. Kousoulas, V. Karagianni, A. Pelekanou, A. Antonopoulou, E.J. Giamarellos-Bourboulis, *(Athens, GR)*

Objectives: Monocytes release pro-inflammatory cytokines leading to inflammatory response syndrome (SIRS). The present study aimed to unravel the differences of monocyte

triggering between SIRS of patients with acute pancreatitis compared to SIRS of patients with sepsis.
Methods: Peripheral blood monocytes were isolated from 25 patients by density gradient centrifugation of whole blood, incubation in RPMI and removal of non-adherent cells. Twelve patients had sepsis and 13 patients acute pancreatitis; in all symptoms presented within 12 hours before admission. After diagnosis 20 ml blood was sampled. Half were assayed for isolation of monocytes and 10 ml were centrifuged for serum estimation of tumour necrosis alpha (TNF-a) and interleukin-6 (IL-6). Half of monocytes were incubated in the presence of 4% of patients' serum and supernatants were collected. The other half was lyzed; caspase-3 was estimate in the lysate by an enzymatic chromogenic assay. TNF-a and IL-6 were estimated in serum and cell supernatants by an enzyme immunoassay.
Results: Median ± SE of TNF-a of serum in septic patients and in patients with acute pancreatitis was 11.61 ± 7.57 and 17.01 ± 8.64 pg/ml, respectively. Respective values of IL-6 in septic patients and those with acute pancreatitis were 192.30 ± 35.40 and 21.00 ± 16.05 pg/ml (p = 0.001). Respective values of intracellular activity of caspase-3 were 0.94 ± 0.17 and 0.34 ± 0.09 pmol/min.104 cells, (p = 0.049). TNF-a of monocyte supernatants did not differ between patients with acute pancreatitis and sepsis, a result that remain unaltered in the presence of patients' serum. IL-6 of monocyte supernatants of patients with sepsis was considerably increased after addition of patients' serum compared to patients with pancreatitis (see Figure).



Conclusion The data have shown that monocyte activity was different between acute pancreatitis and sepsis. The different kinetics of the release of IL-6 might speculate for the existence of a probable anti-inflammatory mechanism active in acute pancreatitis but not in sepsis.

P1179

Recombinant *Escherichia coli* as a model to study a potential pathogenic agent of *Burkholderia pseudomallei*-phospholipase C

B. Sadowska, M. Wieckowska-Szakiel, W. Rudnicka, S. Korbsrisate, B. Rozalska *(Lodz, PL; Bangkok, TH)*

Objectives: *Burkholderia pseudomallei* is the etiological agent of melioidosis – endemic disease, whose acute form is manifested as pulmonary or septicemic infection with abscesses formation. The pathogenic factors of these Gram-negative bacilli are not known well enough. However, it is worth noting, that they produce many enzymes including phospholipase C (PLC). We hypothesised that PLC can be important for *B. pseudomallei* interaction with host immune cells.
Methods: A parental *E. coli* DH5alpha strain (PLC-) and recombinant mutant *E. coli* pSN-1a producing phospholipase C from *B. pseudomallei* (PLC +) were used for the study. The mouse peritoneal macrophages were infected with FITC-labelled and ''normal'' bacteria to determine their adherence to the cells by a fluorimetric assay and ingestion / intracellular killing by a colony forming units-assay. The supernatants were collected to estimate the production of IL-12 and TNF-alpha by infected phagocytes using DuoSet ELISA (RandD).
Results: The adherence of *E. coli* DH5alpha to the macrophages was stronger that of *E. coli* pSN-1a mutant, which also corresponded with better ingestion and intracellular killing of this PLC- strain by used phagocytes. Interestingly, the phagocytic activity of the cells infected with the mixed population of both *E. coli* (PLC + and PLC –) strains was diminished as compared with their activity against the homogenous populations of these bacteria. Two hours incubation of the macrophages with those *E. coli* strains led to the production of TNF-alpha (the level of IL-12 was below detection limit). However, the stimulatory effects of parental and mutant strain as well as their homo- and heterogeneous cultures were similar.
Conclusions: The production of phospholipase C seems to disturb phagocytic but not secretory activity of the macrophages. Thus, our data suggest that this enzyme can play an important role in the survival of *B. pseudomallei* inside professional phagocytes. Some investigators even suggest that phospholipase C of *B. pseudomallei* can play a similar role in the pathogenesis of melioidosis as phospholipase for Listeria monocytogenes, which takes place during the ''escape'' of these bacteria from the phagolisosomes.Supported by Grant No. 3 PO4C 081 24 from State Committee for Scientific Research.

P1180

Fusobacterium necrophorum in young patients with tonsillitis and healthy controls examined by real-time PCR. Concomitant Group C streptococci may aggravate tonsillitis

A. Jensen, L.H. Kristensen, J. Prag *(Viborg, DK)*

Objectives: *Fusobacterium necrophorum* (Fn) is presumably a part of the normal human throat flora. *Fusobacterium necrophorum* subsp. *funduliforme* can cause tonsillitis, peritonsillar abscesses and Lemierre's syndrome. We studied the prevalence of Fn in healthy controls and patients with acute and chronic tonsillitis by a novel real-time PCR assay and analysed the association of Group C streptococci with Fn in tonsillitis patients.
Methods: Throat swabs from 93 healthy controls (age 18–32, median 21 years) and 60 swabs from tonsillitis patients (age 18–32, median 23 years) were examined quantitatively for Fn by a novel Fn subspecies specific real-time PCR assay. Swabs from both groups were simultaneously inoculated aerobically on 5% Columbia blood agar to detect haemolytic streptococci and anaerobic in order to find Fn.
Results: PCR detected that 20% of 93 controls had Fn, and they were all subsp. *funduliforme* in the throat in quantities ranging form approx 1.6×104 to 3.5×105 CFU/swab, mean: 8×104 CFU/swab. No Fn was found in 80%. There were none positive culture with neither Fn nor hemolytic streptococci on the agar plates. Fifty percent of the swabs from 60 tonsillitis patients revealed Fn, again all were subsp. *funduliforme* by PCR in quantities raging from 7×102 to 4.2×106 CFU/swab, mean: 1.2×106 CFU/swab. Only 3 positive PCR samples, (3×105 to 2.5×106 CFU/swab) were positive for Fn when cultured. Both the number of positive samples and the amount of CFU/swab

	Controls	Tonsillitis
Total	92	60
PCR Positive	19	30(1)
Mean CFU/swab	8.0×10^4	$1.2\times10^{6(1)}$
$C_T \leq 32$	0	15(1)
Group C streptococci	0	19(1)
PCR positive	-	11
Mean CFU/swab	-	$2.2\times10^{6(2)}$
$C_T \leq 32$	-	10(2)

(1) Compared to controls ($p < 0,05$).
(2) compared to tonsillitis without Group C streptococci ($p < 0,05$).
C_T = treshold cycle.

were significantly greater in swabs from tonsillitis patients, $p < 0.05$. Patients with Group C streptococci had significantly higher CFU/swab values than those without Group C streptococci, (CFU/swab: 2.5×105 to 3.7×106 mean: 2.2×106 CFU/swab). Results are summarized in table 1.
Conclusion: Fn subsp. *funduliforme* is part of the normal throat flora in small quantities in 20% of healthy controls and in higher quantities in 50% of patients with acute or chronic tonsillitis in young people, indicating that Fn may cause tonsillitis. In addition this study indicates that concomitant Group C streptococci may aggravate tonsillitis.

P1181

Expression, immunogenicity and apoptotic potential of *Salmonella enterica* serovar Typhi 69 kDa phenotype under oxidative stress, iron limitation and anaerobic conditions

V. Chanana, S. Majumdar, P. Rishi *(Chandigarh, IN)*

Objectives: Invasive Salmonella has been reported to induce apoptosis of macrophages as a part of its infection process, which may allow it to avoid detection by the innate immune system. However, the induction of apoptosis remains to be looked into under the different host environments experienced by pathogen. Successful pathogens overcome the host environmental stresses by the coordinated expression of various genes and eventually proteins. Since, the surface of the microbe is likely to come in contact with the host initially an attempt was made to identify the Salmonella outer membrane proteins (OMPs) which may get expressed under more than one environmental conditions simulating the *in vivo* ones and to assess their apoptotic potential.
Methods: *S. Typhi* was grown under iron-depleted, oxidative stress as well as anaerobic conditions and their OMP profiles were compared. The phenotypic similarity among the stresses-induced proteins was assessed on the basis of their similar molecular weight, cross reactivity and HPLC. For apoptosis, assessment of nucleosomal DNA, nuclear staining with acridine orange-ethidium bromide and Hoechst33342-PI co-staining as well as by flow cytometry was done. Estimations of cytokines (TNF-a, Il-6, IL-1a), reactive nitrogen intermediates, lipid per oxidation, levels of antioxidants; superoxide dismutase and catalyse were done.
Results: A 69 kDa OMP was found to express with enhanced intensity under the selected stress conditions in comparison to normal conditions. The protein expressed under anaerobic and oxidative stress reacted with the antibodies raised against iron-regulated OMP, indicating the sharing of at least some of the epitopes. A single peak observed after subjecting the pooled 69 kDa protein sample to HPLC confirmed the purity and phenotypic similarity of this protein. Reactivity of pooled 69 kDa protein with 85% of widal positive sera from typhoid patients revealed its *in vivo* expression. Stress-induced 69 kDa OMP caused apoptotic cell death in 49% of macrophages. A significantly enhanced expression of cytokines (TNF-a, IL-1a and IL-6) was detected when macrophages were interacted with stressed OMP. A significant increase in the levels of oxidants and decrease in antioxidants was also observed.
Conclusion: The findings of the study may be relevant in better understanding of pathophysiology of the disease during the host-pathogen interactions and for future development of diagnostic as well as preventive strategies.

P1182

Gram-positive and Gram-negative bacteria induce different cytokines from human peripheral blood mononuclear cells irrespective of taxonomic relatedness

S. Johansson, A. Bigsten, C. Hessle, A. Wold *(Goteborg, SE)*

Objectives: Upon bacterial stimulation tissue macrophages produce a variety of cytokines that orchestrate the immune response necessary to clear the infection. Earlier studies have shown that Gram-positive bacteria induce higher levels of IL-12, IFN-gamma and TNF from human peripheral blood mononuclear cells (PBMC) than Gram-negative bacteria, whereas Gram-negatives induce much more IL-6, IL-8 and IL-10 than Gram-positives. The aim of this study was to examine if this dichotomy is true for Gram-positive and Gram-negative bacteria irrespective of their position in the taxonomical system, and to relate cytokine production to phagocytosis.
Methods: PBMCs from healthy blood donors were incubated with 37 species of UV-inactivated Gram-positive and Gram-negative bacteria well spread in the taxonomical system. IL-12, TNF, IL-1beta, IL-6 IL-8 and IL-10 were measured in the supernatants after 24 h and IFN-gamma after 5 d with ELISA. Phagocytosis was studied in cytospin preparations after 30 min incubation.
Results: Irrespective of their position in the phylogenetic tree, Gram-positive bacteria induced more IL-12, IFN-gamma and TNF than Gram-negatives, while Gram-negative bacteria were more potent stimulators of IL-6, IL-8 and IL-10 than Gram-positives. IL-1beta was induced in equal amounts from Gram-positive and Gram-negative bacteria. Production of IFN-gamma and TNF were strongly correlated, as were IL-6 and IL-8, and IL-6 and IL-10. There was no difference between Gram-positive and Gram-negative bacteria in the degree of phagocytosis. Nor were there any correlations between cytokine production and bacteria phagocytosed.
Conclusion: The results confirm that Gram-positive and Gram-negative bacteria trigger different cytokine patterns in human monocytes, irrespective of their genetic relatedness. Differences in composition of the bacterial wall are likely to account for these differences.

P1183

Invasiveness of *Providencia alcalifaciens* to HEp-2 cells

A. Maszewska, A. Torzewska, A. Rozalski *(Lodz, PL)*

Objectives: *Providencia alcalifaciens* is a Gram-negative bacterium from the family *Enterobacteriaceae* and is thought to be a cause of diarrhoea in travellers and children. Cells invasion

has been proposed as the main virulence mechanism of this bacterium. The purpose of this study was to determine invasive ability and other virulence-associated characteristics in *P. alcalifaciens* strains.

Methods: Twenty two *P. alcalifaciens* strains isolated from patients with diarrhoea were studied. Invasiveness of *P. alcalifaciens* strains was preliminarily screened in the quantitative HEp-2 invasion assays. The bacterial strains were tested for actin condensation with HEp-2 cells in the fluorescent actin staining test. The roles of bacterial and host factors in invasion were investigated using selective inhibitors that influence specific bacterial or host cell functions. Plasmid profiles were determined by Kado-Liu method followed by horizontal electrophoresis on 0.8% agarose gels. Electrophoretic whole-cell protein patterns of *P. alcalifaciens* isolates were examined.

Results: Of the 22 strains of *P. alcalifaciens* studied, 21 were invasive for HEp-2 cells. All the invasive strains caused actin condensation in infected cells. Inhibition of bacterial protein synthesis decreased dramatically invasion of HEp-2 cells. Tyrosine kinases play an important role in *P. alcalifaciens* uptake into HEp-2 cells. The ability of *P. alcalifaciens* to invade HEp-2 was inhibited by depolymerization of microfilaments and microtubules. Monodansylcadaverine that inhibit receptor-mediated endocytosis, surprisingly enhanced entry of some strains of *P. alcalifaciens* into HEp-2 cells. Endosome acidification did not seem to have any appreciable effect on invasion *P. alcalifaciens*. Plasmid DNA analysis showed the presence of plasmids of 5–172 kb in 13 strains regardless of their invasive ability, suggesting that invasiveness in *P. alcalifaciens* is not plasmid related. Numerical analysis of protein patterns of 22 *P. alcalifaciens* strains based on UPGAMA method, formed two distinct group defined as A and B. Interestingly, more of invasive strains belonged to group A.

Conclusion: These findings define some requirements for the *P. alcalifaciens* entry mechanism. Further studies are needed to precise events that occur during bacterial entry and identify the gene(s) responsible for invasion of these bacteria.

P1184

The exchange of virulence-related and drug-resistance genes among clinical isolates of enterococci

N. Amirmozafari, M. Alebouyeh, H. Forohesh *(Tehran, IR)*

Objectives: Enterococci comprise an important and diverse group of bacteria that cause disease in human and animals. They reside in the gastrointestinal tract of human and animal, soil, water, foods, and can persist in elevated salt contents and various pH values. They can readily acquire antibiotic resistance and various other virulence factors. In this study, the prevalence of various virulence factors among different clinical isolates of enterococci versus those isolated from healthy individuals was compared.

Methods: Enterococcal strains isolated from clinical and healthy cases were tested for various virulence related properties such as haemolysin, gelatinase, hemoglutinin, DNase, and phremone (aggregative substance) production. Their antibiotic resistance patterns were also determined. The ability to exchange resident plasmids via conjugation was tested by two different mating protocols.

Results: The frequency of gelatinase, aggregation substance, and hemolysin production was higher in *E. faecalis* relative to those in *E. faecium*. However, no statistically significant differences were detected in the other traits. Phremone-responsive plasmids were common in most isolates and had the ability to transfer between strains with high frequency. Most isolates contained one or more plasmids in the 3–98 MDa range. Two isolates showed total resistance to all of the antibiotics tested. Antibiotic resistance genes had the ability for conjugational inter-strain transfer. The prevalence of aggregative substance in the strains isolated from clinical cases was much higher than those obtained from the control group.

Conclusion: Since no known exotoxin molecule was identified in enterococci, their pathogenic potential may be attributed to a variety of extra cellular enzymes, antibiotic resistance, aggregative substance, and other factors. Their importance in medicine is related to their ability to acquire antibiotic resistance and cause nosocomial infections in hospitalized and debilitated patients. The statistically significant higher proportion of aggregative substance in enterococci isolated from sick people relative to those obtained from healthy cases, points to the pivotal role conjugational gene transfer may play in the acquisition of pathogenic potential.

P1185

Modulation of GRO-alpha and TNF-alpha production by peripheral blood mononuclear cells treated with killed *Helicobacter pylori*

G. Stassi, A. Arena, A. Cascio, C. Iaria, M. Calapai, D. Gazzara, D. Iannello *(Messina, IT)*

Objectives: To evaluate the *in vitro* production of GRO-a and TNF-a by human peripheral blood mononuclear cells (PBMC) after stimulation with a suspension of *Helicobacter pylori* (Hp) (live, gentamicin-killed or a combination of killed and live). Gastric colonization by Hp is the major cause of chronic active gastritis and is often associated with duodenal and gastric ulceration, gastric carcinoma and mucosa-associated lymphoid tissue lymphoma. GRO-a seems to play an important role in recruiting and activating neutrophils in the gastric mucosa. TNF-a has been demonstrated to up regulate GRO-a production and, at high concentrations, to injure the gastric mucosa.

Materials and methods: PBMC obtained from 5 healthy Hp-seronegative donors were cultured, and, after the stimulation with Hp, supernatants were analysed for the presence of TNF-a and GRO-a by immunoenzymatic methods. Hp isolated from a man with duodenal ulcer was cultured and suspended in PBS to a concentration of 109 bacteria/ml. The combined treatment was performed by adding to PBMC a suspension of gentamicin-killed hp (1.2 x 109 killed bacteria/ml) for 20 hours and after this period adding 1.2 x 109 lives Hp for further 24 h. After 44 h supernatants were harvested, centrifuged and stored at 80°C until cytokine assays. To verify a possible correlation between TNF-a and GRO-a monoclonal antibodies (MAb) anti-TNF-a or anti-GRO-a were added to PBMC in all experimental conditions.Data were analysed by one-way analysis of variance and by Student-Newman-Keuls test.

Results: The amounts of TNF-a and GRO-a produced by PBMC after stimulation with live Hp were higher than those produced after stimulation with a combination of killed and live Hp and the latter were higher than those produced after stimulation with killed Hp. The addition of MAb anti-TNF-a to PBMC respectively treated with live, killed, or killed + live Hp, determined lower levels of GRO-a. These results demonstrate that the strong increase in GRO-a expression in PBMC infected with live Hp was, at least in part, dependent on the presence in supernatants of TNF-a. On the contrary, the addition of MAb anti-GRO-a did not influence the TNF-a release.

ISSN: 1470-9465

Conclusions: Our data show that pre-treatment with killed Hp can decrease inflammatory response during Hp infection. The hypothesis that killed Hp could influence the outcome of Hp infection requires further investigation.

P1186

Elevated sialidase and prolidase combined with vaginal pH > 5 are biomarker for low birth weight and early preterm birth

S. Cauci, J. McGregor, P. Thorsen, J. Grove, D. De Santo, F. De Seta, P. Lanzafame, S. Guaschino *(Udine, IT; Los Angeles, US; Aarhus, DK; Trieste, IT)*

Introduction: Preterm birth is a multifactorial condition. Infections are responsible for about 50% of early preterm birth. Vaginal infections especially bacterial vaginosis have been associated with adverse pregnancy outcomes.
Objective: Assess if easy to measure vaginal fluid biomarkers are predictive for low birth weight (LBW, < 2500 g), very LBW (VLBW, < 1500 g), spontaneous preterm at < 37 weeks gestation, and total preterm deliveries (at < 37, < 35, < 32 weeks gestation).
Methods: Low and high cut-offs for vaginal fluid pH, sialidase and prolidase activities were examined in a nested case-control study of 579 Danish women (from a study population of 2,846 women) with samples collected at mean 17 weeks gestation. 116 LBW (17 VLBW), 117 preterm deliveries (85 spontaneous), and 418 normal term deliveries were analysed.
Results: Vaginal pH ≥ 4.7 or pH ≥ 5 by itself was not associated with LBW or prematurity. Conversely, combination of pH ≥ 5 and high sialidase activity demonstrated OR 17 (CI 1.8–150) for LBW; OR 31 (CI 1.8–516) for VLBW; along with OR 18 (CI 1.6–204) for preterm at <35 weeks and OR 31 (CI 1.9–542) for preterm at < 32 weeks gestation. The combination of pH ≥5 and high prolidase activity demonstrated OR 13 (CI 1.3–122) for LBW; OR 33 (CI 2.0–553) for VLBW; as well as OR 9.2 (CI 0.6–150) for preterm at < 35 weeks and OR 35 (CI 2.0–586) for preterm at < 32 weeks gestation. In this population, no woman having high sialidase and high prolidase activity had a term birth, or a baby weighting ≥2500 g at birth.
Conclusion: In this Danish population, mid gestation findings of vaginal fluid elevated pH with sialidase and/or prolidase were associated with LBW, VLBW, and early preterm at < 35, or < 32 weeks gestation.

P1187

Levels of IgA against *Gardnerella vaginalis* hemolysin (anti-Gvh IgA) before and after therapy of bacterial vaginosis

S. Cauci, S. Driussi, G. Casabellata, M. Di Santolo, P. Lanzafame, D. De Santo, F. De Seta, S. Guaschino *(Udine, IT)*

Objectives: Bacterial vaginosis (BV) is a complex change of the vaginal ecology, its pathogenesis is still elusive. *Gardnerella vaginalis* is virtually always present in the abnormal flora milieu of women with BV.So far, the only characterized vaginal specific IgA response in women with BV, is the IgA against the hemolysin produced by *G. vaginalis* (anti-Gvh IgA).The anti-Gvh IgA appears to be a critical host response in vaginal fluid of women with BV. Interestingly, a study performed in Caucasian pregnant women showed that high anti-Gvh IgA levels were protective for preterm birth and low birth weight. On the contrary, low anti-Gvh IgA values associated with high microbial hydrolytic enzymes (sialidase and prolidase activity) were correlated with increased risk of adverse pregnancy outcomes including early preterm birth (< 32 weeks gestation) and very low birth weight. To our knowledge, no study has evaluated the anti-Gvh IgA response before and after therapy of BV.The aim of the study was to assess changes of anti-Gvh IgA in vaginal fluid before and after antibiotic treatment of women with BV.
Methods: Enrolled women were aged 18–50 years. A 7 days oral metronidazole therapy was administered in accordance with CDC protocols for fertile women with BV (4 Amsel's criteria positive and Nugent score 7–10). Patients were re-evaluated 1 month after starting of the antibiotic treatment. Anti-*G. vaginalis* hemolysin IgA were evaluated in the vaginal fluid by ELISA according to a procedure previously described, values were obtained as absorbance in millioptical density units (mOD) at 405 nm.All measurements were performed in duplicate.The Mann-Whitney U-test was used to compare anti-Gvh IgA levels between groups. Any P value < 0.05 was considered statistically significant. The software package SPSS (Statistical Package for Social Sciences) was used for data analyses.
Results: Anti-Gvh IgA values were largely increased (3-fold, P = 0.001) after therapy in women cured by the antibiotics.On the opposite, levels of vaginal anti-Gvh IgA were unaffected by the antibiotic treatment in women not cured (still having BV) .
Conclusions: It is presently not known why the currently adopted antibiotic treatments for BV give a low cure rate (around 60–80%). A major problem for clinicians is to evaluate if the woman is cured after therapy as vaginal flora is altered by antibiotics. Rise of vaginal anti-Gvh IgA is a reliable objective marker to assess cure of BV after antibiotic treatment.

P1188

Q fever in Northern Greece: epidemiologic and clinical data from 58 acute and chronic cases

M. Pape *(Thessaloniki, GR)*

Objective: The clinical presentation of *Coxiella burnetii* is very pleomorfic and non-specific. The incidence of Q fever among humans is probably underestimated, and diagnosis relies upon the physician's awareness of the symptoms of Q fever and the presence of a reliable diagnostic laboratory. The aim of this study was to investigate the epidemiologic and clinical aspects of acute and chronic Q fever in northern Greece.
Methods: Over a period of 2 years (2004–2005), 980 patients (520 men, 460 women, mean age 45 ± 5 yr) were examined and 58 cases of Q fever (6%) were identified. The diagnosis was based on clinical manifestations (flu-like syndrome, pneumonia, hepatitis, pericarditis, lympathenopathy, Guillain-Barre, endocarditis) along with serological confirmation (ELISA/IFA methods). Acute Q fever was diagnosed on the basis of phase II IgG and IgM titers > 1:256 and 1:40 respectively. Chronic Q fever was suspected on the basis of prolonged disease and the presence of phase I IgG titer ≥ 800.
Results: 65% of the patients were men (38/58) and 35% were women (20/58). We classified patients according to the different clinical forms of acute and chronic Q fever: pneumonia (28-cases), flu-like syndrome (15-cases), hepatitis (7-cases), lymphathenopathy (2-cases), pericarditis (2-cases), Guillain-Barre (1-case), osteomyelitis (1-case), optic neuritis (1-case). Hepatitis occurred in younger people and pneumonia in older and more immunocompromised patients. Contact with animals was found to be a major risk factor for acquisition of Q fever, since 40 patients (68%) had a history of contact, mainly with sheep (30 patients: 51%). Only one chronic case, an endocarditis

fulfilling the Duke criteria (IgG class anti-phase I titre > 800) was detected. Most patients were treated with doxycycline and recovered. Only two patients who were significantly older and more immunocompromised than the others died eventually.
Conclusion: Q fever was rarely notifiable disease in Northern Greece. Our data indicate, however, that Q fever should be considered a public health problem with several clinical manifestations.

P1189

Gram-negative commensal gut bacteria aggravate small intestinal inflammation via toll-like receptor 4

M.M. Heimesaat, A. Fischer, H.-K. Jahn, J. Niebergall, O. Liesenfeld, R.R. Schumann, S. Bereswill, U.B. Göbel *(Berlin, DE)*

Gram-negative commensal gut bacteria such as *Escherichia coli* and *Bacteroides* sp. are suspected to contribute profoundly to inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. It has been recently shown that both bacterial groups accumulate at very high levels in the inflamed ileum and trigger severe Th1-type immunopathology of acute pan-ileitis in susceptible mice infected perorally with 100 cysts of *Toxoplasma gondii*. Thus, this model is excellently suited for the study of host-bacterial-relationships in small intestinal inflammation. To unravel the mechanisms by which bacteria aggravate ileal inflammation, we investigated the severity of small intestinal inflammation in mice lacking Toll-like receptors (TLRs) 2 and 4, which constitute important components of the bacteria-related innate immune system. *Escherichia coli* and *Bacteroides* sp. accumulated to comparable levels during ileitis and their concentrations did not differ significantly in inflamed ilea of wild type mice and of mice lacking TLR2 or TLR4. However, the severity of ileitis was clearly diminished in mice lacking TLR4-/-, as indicated by reduced histological scores and letality. Because TLR4 is highly specific for bacterial lipopolysaccharide (LPS), these findings provide evidence that commensal gut bacteria aggravate the immunopathology of intestinal inflammation via TLR4-mediated sensing of bacterial LPS, which might play a key role in inflammatory process.

P1190

The condition of a mucous stomach membrane of region blood-groove after shigellosis

M. Chemich, A. Snitsar *(Sumy, UA)*

Objectives: To study a mucous stomach membrane of region blood-groove (MSMRB) in the course of late reconvalescation of shigellosis (Sh), to investigate these changes' dependence on the therapy of the sharp period.
Methods: In the course of late reconvalescation 29 persons have been examined. All of them have suffered middle-severe Sh. and have received the standard treatment: enterosorbents-12 (I group); enterosorbents and one antibacterial medicament-7 (II group); two antibacterial medicaments-10 (III group). MSMRB has been defined on clirens hydrogen in cardiac portion (CP), an average third of body (B) and pyloric portion (PP) of a stomach.
Results: Full normalization of MSMRB in all portions of a stomach was defined in I group of patients: CP - (35.59 ± 2.03), B - (71.18 ± 4.08), PP - (77.73 ± 4.47) ml/min 100 gr. I group of the patients has been got positive dynamics of MSMRB in the course of treatment: CP - (22.07 ± 2.83), B - (43.69 ± 5.60), PP - (48.11 ± 6.16) ml/min 100 gr. The lowest and the poorest parameters of MSMRB, in terms of prognosis, have been received in III group of examined: CP - (21.24 ± 2.33), B - (43.12 ± 4.73), PP - (46.98 ± 5.15) ml/min 100 gr. The patients blood-groove was below age norm in all portions of a stomach: (32.18 ± 2.53), (64.36 ± 4.06), (70.79 ± 4.76) ml/min 100 gr accordingly. It did not differ from the data received in the course of hospitalisation. MSMRB in this group was also considerably below data of all examined and groups of patients who received enterosorbents (Ð < 0.05–0.001). Direct strong correlation between a degree of MSMRB recovery is revealed and therapy that has been performed in the sharp period - enterosorbtion or antibacterial therapy.
Conclusion: In the course of late reconvalescation of shigellosis normalization of MSMRB in all portions of a stomach occurred to the examined in whose treatment enterosorbents have been used, or enterosorbent and an antibacterial medicament. The usage of two antibacterial medicaments in the sharp period of treatment influenced MSMRB negatively.

P1191

In vitro model to analyse the effect of mycobacteria infection on HIV replication

A. Boluda, R. Delgado, M.D. Folgueira *(Madrid, ES)*

Objectives: Macrophages are important cellular targets for both HIV and mycobacterial infections. *In vitro* HIV infection of human differentiated macrophages is difficult and usually required the addition of colony-stimulating factors that can mask the role of *Mycobacterium* spp. confection on viral replication. In this study we have developed an infection model using recombinant retroviruses to analyse the role of *Mycobacterium* spp. infection and the effect of diverse cytokines on HIV replication in human macrophages.
Methods: A retroviral vector based on PNL4-3 luc/gfp R-E- pseudotyped with vesicular stomatitis virus glycoprotein (VSV-G) was used to infect macrophages differentiated from peripheral blood monocytes (MDM) by culture for 7 days in medium containing human serum; 24-well plates containing 5 x 10 5 cells/well were infected with the recombinant retrovirus at MOI 1:100. After 5 days the cells were incubated with 7 M. tuberculosis strains (H37 Rv and 6 clinical isolates that presented different susceptibility patterns), *M. avium* and *M. fortuitum* in a 1:1 ratio. Several cytokines (IL-1b, IL-4, IL-10, TNF-a, IFN-g) and sonicates from *M. tuberculosis, M. avium* and *M. fortuitum* ATCC strains were also tested. After 72 h of stimulation or mycobacterial infection the effect of the cytokines and *Mycobacterium* spp. on HIV replication was quantified measuring luciferase activity or gfp expression. Each experiment was made on cells from at least three different donors. Statistical analysis was performed using one-way ANOVA test.
Results: The percentage of macrophages infected using recombinant retrovirus was higher than 50%. The confection with all Mycobacterium strains tested induced an increase on HIV replication (from 1.58-fold to 4.2 -fold) that only presented statistical significance ($p < 0.05$) when the macrophages were coinfected with *M. tuberculosis* strains. Proinflamatory cytokines IL-1b, TNFa and IFNg showed a significant effect on HIV replication. Cell stimulation with Mycobacterium sonicates augmented HIV replication at lower level than coinfection with viable bacilli.
Conclusions: The HIV infection model described in this study allowed to get a high grade of HIV infection in human macrophages and to study the effects of cytokine production driven by mycobacteria and *Mycobacterium* spp. coinfection on

HIV replication. Proinflamatory cytokines IL-1b, TNFa and IFNg and coinfection with *M. tuberculosis* strains increased significantly HIV replication.

P1192

Prevalence of non-tuberculosis mycobacteria in Southeast of Iran

T. Naserpour Farivar, B. Sharifi Moud, M. Naderi, M. Salehi, N. Salari, N. Naserfar *(Zahedan, IR)*

Objectives: Tuberculosis is one of the most important problems in health and treatment in the entire world and especially in the developing countries. Due to the importance of Tuberculosis and its spread and with respect to the high incidence of pulmonary tuberculosis in Sistan and Baluchestan which has the highest rate of Tuberculosis in Iran and due to drug resistance characteristics of Non Tuberculosis Mycobacteria (NTM) to the first line of anti TB drugs and with respect to this point that NTM can produce diseases which may be similar to Tuberculosis, we decide to study the prevalence of isolation of NTM in this area which undoubtedly has special value on treatment decision and present diagnostic procedure.
Materials: This study was done in two different years in 2000 and 2004 in patients referring to BOU-Ali hospital with pulmonary symptoms which had a smear positive in ZNCF staining and positive culture results in cultivation on Lowenstein-Johnson and Middle brook 7H10 agar. The isolated mycobacteria were identified on the basis of their growth rate, pigmentation and biochemical testes.
Results: In our 2000 study, the results of cultural characteristics(i.e. growth rate and pigmentation) and biochemical testes in 91 specimens (60.7%) lead to isolation of Mycobacterium tuberculosis and in 59 specimens (39.3%) to isolation of NTM. In NTM isolates,38 specimens (64.4%) belongs to females and 21 specimens (35.6%) belongs to males. In our 2004 study, the results of cultural characteristics (i.e. growth rate and pigmentation) and biochemical testes in 60 specimens (66.6%) lead to isolation of Mycobacterium tuberculosis and in 20 specimens (33.3%) to isolation of NTM and from these 20 NTM specimens, 13 specimens (65%)belongs to females and 7 specimens (35%) belongs to males. In our both studies (2000 and 2004), the most amount of isolation of NTM was in upper 60 years old group.
Conclusion: Due to reported anti TB drugs resistance of NTM and with respect to our findings, it is suggested that in the first visit of patients suspected to tuberculosis, simultaneous study of smear staining, cultivation, species identification and antibiogram, specially in this region and in similar geographic conditions be done and treatment with the results of ZNCF staining alone may be not a suitable diagnostic test.

P1193

Systemic inflammation in the field of experimental multiple trauma: when does bacterial translocation and subsequent sepsis occur?

P. Koutoukas, T. Tsaganos, N. Efstathopoulos, K. Panousis, P. Kaldis, M. Klonaris, E.J. Giamarellos-Bourboulis, A. Papalois *(Athens, GR)*

Objectives: To evaluate the role of bacterial translocation in the sequences of events occurring in multiple trauma.

Methods: A total of 32 New Zealand rabbits were applied divided in three groups as follows: A (n: 10), controls; B (n: 11), rabbits subject to myotomy of the right femur; and C (n: 11), rabbits subject to myotomy followed by crush injury of the right femur. Blood was drawn at 0 time and at 2, 4, 24 and 48 hours following intervention. Endotoxins (LPS) were estimated by the QCL-1000 LAL assay, blood bacterial counts after quantitative blood culture and tumour necrosis factor-alpha (TNF-a) by a bioassay on L929 fibrosarcoma cell line. Apoptosis of lymphocytes and monocytes was estimated in heparinized whole blood after red blood cell lysis, incubation of white blood cell with ANNEXIN-V and propidium iodide and subsequent passage by a flow cytometer.
Results: Results are given as medians in the Table (*counts of *Pseudomonas aeruginosa*).

Time (h)	Lymphocyte apoptosis (%)	Monocyte apoptosis (%)	LPS (EU/ml)	$\log_{10}$ of bacteria (cfu/ml)*	TNFα (pg/ml)
			Group A		
2	0.55	11.65	2.77	1.69	5.75
4	1.41	25.56	0.50	1.69	186.71
24	13.35	17.42	0.50	1.69	38.45
48	1.98	55.32	0.50	1.69	95.14
			Group B		
2	0.46	3.49	2.52	1.69	19.86
4	35.27	27.98	0.52	2.38	164.75
24	9.42	39.85	0.50	1.69	64.47
48	1.06	22.35	0.50	1.69	11.50
			Group C		
2	14.69	40.60	0.67	3.97	229.50
4	13.69	48.57	0.69	3.43	143.96
24	7.87	26.93	0.50	1.69	88.25
48	8.81	27.24	0.50	1.69	28.04

Conclusions: Multiple traumas as applied in experimental model are accompanied by early translocation of bacteria and subsequent apoptosis of lymphocytes and monocytes. The phenomenon is aggravated in relation to the degree of injury from muscle injury to bone crush.

P1194

Identification of *Borrelia burgdorferi* sensu lato species in erythema migrans and cardial involvement by real-time PCR and electron microscopy

D. Hulinska, M. Bojar, V. Hulinsky, P. Bartunek *(Prague, Nymburk, CZ)*

Background: *Borrelia garinii, B. afzelii, B. burgdorferi* s.s. and recently *B. valaisiana* and *B. spielmanii* have been isolated from humans. PCR typing methods are usually able to directly detect in human samples three of these species only. Based on real time PCR and on sequencing of the OspA gene we overcame problems of sensitivity and specificity of PCR.
Materials and methods: Forty-nine patients of group 1 had erythema migrans (EM), 21 patients of group 2 had cardial involvement after EM and 17 patient were positive in BSK II medium from tissue, blood and CSF We used direct sequencing of OspA and OspC products by the dideoxy chain termination procedure and quantitative analysis by using CEQ 200XL sequencer and LightCycler real-time PCR (Roche).
Results : *B. garinii* was prevalent in the tissue of the inner organs, heart, synovium, lung, brain. Electron microscopy showed Borrelia in connective tissue far away of the inflammation. *B. afzelii* was prevalent in the skin. Thirty five percent of *B. afzelii*, 16.3% of *B. burgdorferi*, 22.4% of *B. garinii*, and 4% of *B. valaisiana* were detected in patients of group 1. Predominant causative agent in group 2 was *B. garinii*, OspA-type 4.5 (26.3%) and *B. valaisiana* (14.2%) but only *B. burgdorferi* s.s. was cultivated from heart. All genospecies were isolated in

BSK II medium. Sequence OspA of *B. bissetti*- like and A14S genospecies were found in ticks and blood. Quantitative data, distances in phylogenetic trees showed differences not only between OspA or OspC subtypes but also between wild Borrelia and cultured strains. Further studies on the prevalence of this species in clinical materials and ticks are necessary to clarify its pathogenesis.

Partially supported by 6th Frame work Program of the European Community-BOVAC.

P1195

Seroepidemiological study on toxoplasmic infection among high-school girls by IFAT test in Iran, 2002–2003

M. Mahmoudi *(Esfahan, IR)*

The purpose of this study was determination of the positive and ascending serological titer of toxoplasmic infection in high-school girls and effectiveness of some relevant factors.This descriptive-analytic survey was performed with 414 sera collected from High –School girls of six region of Esfahan city by cluster random sampling method. The specimens were taken from the tip of the finger by haematocrite microtubes. The samples were studied by Indirect Immunofluorescent Assay Test (IFAT) for the estimation of serological titers. Data were analysed by two statistical methods: as (χ^2 and t test).In this study, the overall sero – positive rate was 18.4% in 14–19 years old girls. There was an increase in positivity with increasing the age. IFAT titer of 98% of the positive samples was 1:100 and remaining 2% were more than 1:100 to which were performed another titration test to retrieve exact titer. Possible effective risk factors were as follows: Age, region of living, educational factor (parents and student), consumption of undercooked meat and raw liver, occupation and their parents income, exposure to contaminated sources such as cats and poultries. The most contaminated group was in the region number One with prevalence of 27.5% and the least one was in the region number Two with prevalence of 14.5%. Significant differences were found in seropositivity and the exposure with cat and also keeping poultry in the house (P > 0/05). No significant difference was demonstrated in the seropositivity and the other factors. There was a low level of knowledge about toxoplasma and toxoplasmosis and relevant factors. Only 2.4% of the girls were relatively aware regarding this subject. There was not any acute case.

SERO EPIDEMIOLOGICAL STUDY ON TOXOPLASMIC INFECTION AMONG HIGH SCHOOL GIRLS BY IFAT ACCORDING TO REGIONS OF STUDY IN ESFAHAN CITY, IRAN.

	Seropositive	Seronegative	Total
Region no 1	14 27.5%	37 72.5%	51 100%
Region no 2	16 15.4%	88 84.6%	104 100%
Region no 3	14 22.2%	49 77.8%	63 100%
Region no 4	14 19.2%	59 80.8%	73 100%
Region no 5	9 14.5%	53 85.5%	62 100%
Region no 6	9 14.8%	52 82.2%	61 100%
Total	76 18.4%	338 81.6%	414 100%

Conclusion: Toxoplasmic infection is very important because of its socio-economic aspects, therefore control measurements must be performed. All seronegative women should be aware about this infection and its transmition routes. Education is the most important way to prevention and must be progressed via the mass media, training systems and administrations. The study group in this survey were girls in marriage or premarriage ages and it is suggested that managers should continue to offer education about practices that help prevent these kinds of diseases as well as information about preventing toxoplasmosis specifically.

P1196

The faith of *Shigella* species during contact with the water-borne free-living amoeba *Acanthamoebae castellanii*

A. Saeed, H. Abd, G. Sandström *(Stockholm, SE)*

Objectives: Shigellosis is a global human health problem, especially in developing countries with substandard hygiene and unsafe water supplies. Shigellosis is caused by different species of Shigella and the only reservoir known is human. Shigella can be found in natural water in which also free-living amoebae are inhabitants. The aim of this study is to obtain knowledge about the interaction between one water borne free-living amoeba *Acanthamoeba* and *Shigella* species.

Methods: Bacteria and amoebae were co-culture for 21 days and viable counts of both microorganisms were performed. Intra-amoebic growth of bacteria was estimated by gentamyacin assay, and the faith of bacteria in amoeba was judged by microscopy.

Results: The results show that *S. sonnei* multiplies and survives inside trophozoites and cysts of *A. castellanii* and that the bacterial internalisations occur in the cytoplasmic compartment of the amoebae cells. On the contrary *S. flexneri* and *S. boydii* inhibit the growth of *A. castellanii* after three days. Thus, *S. sonnei* and *A. castellanii* seem to have a symbiotic relation, where *S. flexneri* and *S. boydii* show predation.

Conclusions: Here we found that *S. sonnei* may have Acanthamoebae as an environmental host, a finding that did not included other *Shigella* species such as *S. flexneri* and *S. boydii*.

P1197

Mannose-rich capsular polysaccharides enhance uptake and killing of *Klebsiella pneumoniae* by human plymorphonuclear leukocytes

H. Sahly, Y. Keisari, U. Stallforth-Siegel, I. Ofek *(Kiel, DE; Tel Aviv, IL)*

Objectives: Klebsiella capsular serotypes differently stimulate polymorphonuclear leukocytes (PMNL) in a complement-dependent manner but the molecular basis for this distinct interaction is not clear. The aim of this study was to test the relationship between the molecular structure of defined *Klebsiella serotypes* and the induction of complement-mediated respiratory burst in PMNL.

Methods: We employed capsulated and non-capsulated variants as well as capsule-switched derivatives of *K-serotypes* bearing [K21a, K36, K50, K2 (K21a), K2 (K36)] or lacking [K2, K8, K55, K21a (K2)] the Man-alph2/3Man or Rha-alpha2/3Rha sugar repeats, and express either mannose-rich (O3) or mannose-poor LPS (O1) to test their ability to induce complement-dependent respiratory burst and survive in PMNL. Respiratory burst was quantified by measuring the luminol-enhanced chemiluminescence (CL) induced by the bacteria.

Results: The K2, K8 and K55 *K-serotypes* induced significantly lower CL-responses in PMNL than the capsular serotypes K21a, K36 and K50. Similarly, the capsule-switched derivatives K2 (K21a) and K2 (K36), that exhibited Man-alpha2/3Man or Rha-alpha2/3Rha containing capsular polysaccharides but retained the K2 genetic background, induced significantly higher CL-responses than the K2 parental strain. Inversely, the K21a (K2) capsule-switched derivative that expressed the K2 capsular polysaccharides stimulated the PMNL significantly less than the K21a parental strain. The CL-responses induced by non-capsulated variants were significantly lower than those of the capsulated parental strains, suggesting that the contribution of LPS O-antigen is independent from the mannose-content of their O-antigen. The intracellular survival of *K-serotypes* bearing the Man-alpha2/3Man or Rha-alpha2/3Rha sugar repeat (K21a, K36, K50), was significantly higher than that of capsular serotypes lacking these repeats (K2, K8, K55). Depletion of c1q from serum did not affect the CL-response induced by K21a, whereas factor B-depletion revealed a significant reduction of the CL-response not exceeding 60% of that induce by bacteria opsonised by normal serum. EGTA significantly reduced the CL-responses but the values were higher than those induced by K21a opsonised with factor B-depleted serum. The results indicate a capsular Man-alpha2/3Man or Rha-alpha2/3Rha pattern recognition by the complement lectin pathway that leads to enhanced interaction between the capsulated Klebsiella and PMNL.

ISSN: 1470-9465